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A study of the boron - curcumin system

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A Study Of The Boron - Curcumin System

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TABLE OF CONTENTS

INTRODUCTION

The discovery of the usefulness of curcumin for detecting boron was made over 100 years ago. Since that time, a great deal of work has been done in developing new methods based on this compound and in modifying and improving existing methods. However, very little has been done to determine the mechanism of the reactions involved or the structures of the compounds formed.

The purpose of this work was to gather evidence in support of the mechanism proposed in 1956 by Powell, Hardcastle and Poindexter (16) for the formation of the boron - oxalic acid - curcumin complex (Rubrocurcumin) in the Naftel procedure for boron determination. This was done by investigation of the structures of rubrocurcumin and rosocyanin (the product of the reaction between boric acid and curcumin in the absence of oxalic acid) through the determination of their molecular weights and by investigation of the molar ratios of the components of the complexes. A Mechrolab Model 301 Vapor Pressure

Osmometer was used for the work reported in this paper.

HISTORICAL

Curcumin, 1,7 - bis (4 hydroxy - 3 methoxyphenyl) 1,6 - heptadiene 3,5 dione, is the coloring agent in the dye turmeric which is obtained by grinding the rhizomes of curcuma. According to the literature, extraction from turmeric is the most important commercial method of producing curcumin. It is an orange yellow crystalline compound melting at 183°c which has a molecular weight of 368.4 and an empirical formula of $C_{12}H_{20}O_6$.

A derivative of acetylacetone, curcumin exists in two tautomeric forms:
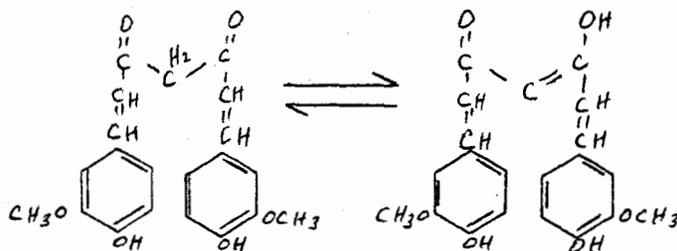
The structure of curcumin has been confirmed by several investigations including those of Ghosh (7), Spicer and Strickland (20), and Silverman and Trego (19).

The discovery of the usefulness of curcumin for

detecting boron was apparently made accidentally in 1821 by Desfosses, who found that a red spot developed on a piece of filter paper soaked in a turmeric solution when a test sample containing boric acid was added (1). Several spot tests for boron based on this discovery have been developed (1,2).

Hebebrand (11) is generally credited with the first colorimetric method for boron based on the turmeric reaction. This method involved the measurement of the red color developed on addition of curcumin to a water – ethanol solution containing boron and hydrochloric acid. Cassal and Gerrans (3) increased the sensitivity of Hebebrands method by adding oxalic acid to the system, and Hegedus (12) later modified the Cassal and Gerrans procedure by combining the oxalic acid and curcumin into one solution in glacial acetic acid.

In 1939, Naftel modified the Cassal and Gerrans method by evaporating the sample to dryness with a solution of $Ca(OH)_2$ before adding curcumin and oxalic acid and evaporating to dryness in ethyl alcohol. The purpose of the calcium hydroxide is to prevent loss of boron as ethyl borate.

In the early 1960's improved methods for determining boron in aqueous samples were developed. Prior to this time, an evaporation to dryness was required in order to

determine these samples by the curcumin - boron complex method. Hayes and Metcalfe (9,10) and Elwell and Wood (6) have reported procedures by which 0.2 ppm of boron can be determined in aliquots which can contain up to 0.25 ml of water in the final volume of 10 ml.

By treating an aliquot of an aqueous sample with sufficient acetic anhydride to react with about 90% of the water present prior to adding curcumin, Crawley (5) was able to determine boron by measuring the intensity of the rosocyanin color which developed within 5 minutes.

At the time Schlumberger (18) isolated and named rosocyanin, he reported that it contained no boron. This was supported by the work of Clarke and Jackson (4) who, on the basis of molecular weight determinations, suggested that rosocyanin was actually an isomer of curcumin with an empirical formula of $C_{14}H_{14}O_4$. Note however, that the empirical formula for curcumin is $C_{12}H_{20}O_6$.

Spicer and Strickland (20) disagreed with the above conclusion, claiming that Schlumberger had used impure curcumin and that Clarke and Jackson had assumed an incorrect formula for curcumin. They found by elemental analysis and by molecular weight determinations that rosocyanin is composed of two molecules of curcumin to one molecule of boron. Their molecular weight determinations were made

cryoscopically in phenol, and it should be pointed out
that the $\triangle T$ which they observed was only about 0.05°C.
Therefore, the accuracy of their method is somewhat
questionable.

Several workers have reported, however, that rosocyanin
is a 1:1 complex. Notable among these are Korenman (13),
Roth and Miller (17), and Silverman and Trego (19).
Roth and Miller obtained evidence in favor of a 1:1 complex
for rosocyanin by preparing curcumin complexes with four
substituted boric acids. The complexes which they prepared
had the following general formula:
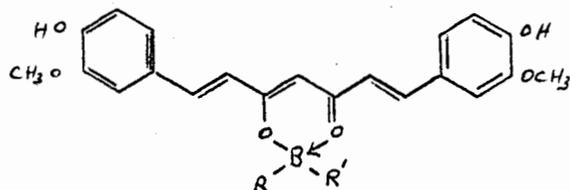
where R and R' were the three possible combinations of
phenyl and 2 - thienyl groups and in one case R and R'
corresponded to 10 - boraxanthydrol.
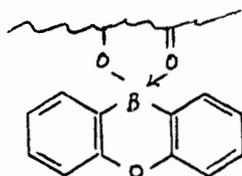
Rubrocurcumin, the complex formed by the reaction of
boron, curcumin and oxalic acid in the Cassal and Gerrans (3)
and Naftel (14) procedures, was named by Clarke and Jackson (4).
They made no attempt however to determine its structure.

Spicer and Strickland (20) showed by elemental analysis that rubrocurcumin exists in the molar ratio of 1:1:1. They suggested very tentatively as a result of observations of its chemical characteristics that rubrocurcumin might exist as a dimer:
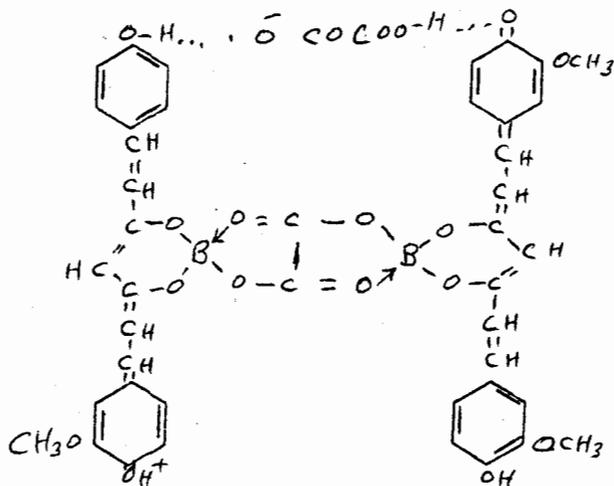
Spicer and Strickland attempted to determine the molecular weight of rubrocurcumin by the cryoscopic method but they reported that their results were erratic and apparently low. They suggested that this might be due to the formation of hydrated rubrocurcumin.

Gol'tman (8) reported that he agreed with the structure suggested by Spicer and Strickland as a result of his investigation of the chemical composition and determination of the maximum absorbance of ethanol solutions of the three components of the complex. These investigations, however, would only show the molar ratio of the components and would not show whether the actual structure of the complex

was 1:1:1 or 2:2:2.

Roth and Miller (17) and Powell, Hardcastle and Poindexter (16) have reported that rubrocurcumin is formed by a 1:1:1 complexation of the form:
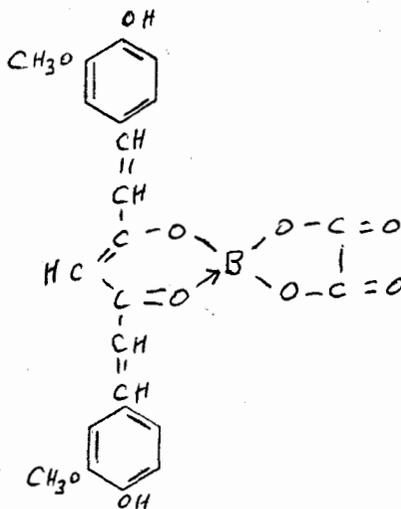
As stated earlier in this paper, the purpose of this research project was to obtain information to help support the mechanism proposed by Powell and co-workers for the formation of this complex in the Naftel procedure. They proposed that, as the solution evaporates, boric acid and oxalic acid react forming a 1:1 complex thus making it easier for the curcumin to enter into the complex.

EXPERIMENTAL

I. Reagents

Reagent grade chemicals were used wherever possible.
In some cases the reagents were dried with anhydrous
$CaCl_2$.

II. Apparatus

Mechrolab Model 301 Vapor Pressure Osmometer

III. Preparations

A. Preparation of Rubrocurcumin

The rubrocurcumin used in this work was prepared by
allowing a solution of boric and oxalic acids and curcumin
in acetone or alcohol with a few drops of HCl to evaporate
to dryness. The solid mass was then powdered with a
mortar and pestle and Soxhlet extracted for six to eight

hours with water and then overnight with acetone. The rubrocurcumin left in the extraction thimble was then recrystallized by dissolving in molten phenol and pouring the solution into benzene, according to the method of Spicer and Strickland (20).

B. Preparation of Rosocyanin

Rosocyanin was prepared by the method of Spicer and Strickland (20). A mixture of boric acid and curcumin was dissolved in absolute alcohol, a few drops of concentrated H_2SO_4 added, and the solution refluxed for three hours. The solution was then poured into water and the crystallized rosocyanin was separated by suction filtration. The rosocyanin was then Soxhlet extracted with ether until colorless and recrystallized by dissolving in ethanol and pouring the solution into benzene.

IV. Description of the Vapor Pressure Osmometer

A Mechrolab Model 301 Vapor Pressure Osmometer was used for the work reported in this paper. This is a simple and very sensitive instrument for measuring small differences in vapor pressure between a solvent and a solution. It consists of a Wheatstone Bridge circuit in which two of the resistors are matched thermistor beads

housed in a thermostated chamber saturated with solvent
vapor. By means of syringes which protrude into the
chamber, a drop of solvent is placed on both the reference
thermistor bead and the measuring bead; and, since these
two drops have the same vapor pressure, the null meter is
zeroed using the balance potentiometer. A drop of the
sample solution is then placed on the sample bead; solvent
condenses on the solution drop due to its reduced vapor
pressure, warming the thermistor bead and causing a bridge
imbalance. The bridge is then rebalanced using a decade
resistance box in series with the sample bead.

The reading on the decade box ($\triangle R$) is proportional to
the term T_2-T_1 in the Clausius - Clapeyron equation

$$T_2 - T_1 = \frac{RT^2m}{\triangle Hv1000}$$

and therefore is proportional to the molar concentration of
the solution (m). The instrument is capable of measuring
temperature differences between the two thermistors as
small as .0001°C.

A calibration curve of $\triangle R$ vs moles/liter is plotted
using a standard of known molecular weight. Benzil was
used as the standard in all of the work reported in this
paper. Since the instrument works on a colligative
principle, the molar concentration of the unknown sample
can be obtained from the calibration curve by determining

$\triangle$R in the same solvent. The molecular weight is then
calculated by:

$$\text{Mol. Wt.} = \frac{\text{grams/liter}}{\text{moles/liter}}$$

V. Preliminary Investigations

A. Solubility Studies

The lowest concentration for which the Vapor Pressure
Osmometer will give reproducible readings is about 0.005
moles/liter. The solvent used must also have sufficient
volatility that it will reach equilibrium with its
surroundings in a reasonable period of time. Therefore,
in order to use the VPO to determine the molecular weight
of rubrocuroumin and rosocyanin, it was necessary to find
a solvent with a reasonably high rate of evaporation in
which the compounds are soluble to the extent of at least
0.005 moles/liter.

Acetone was the only solvent for which quantitative
solubility measurements were made. The solubility of the
complex in acetone was determined by heating to boiling
a sample of an excess of the complex in acetone and
allowing it to evaporate with frequent stirring until the
volume had decreased to about 1/2 of its original volume.
Four 25 ml aliquots of this sample were then pipetted

into tared evaporating dishes and allowed to evaporate to dryness. The dishes were then dried and weighed.

The solubility of rubrocurcumin in acetone, as determined by this procedure is 0.936g/l. Assuming a molecular weight of 468, this corresponds to 0.0020 moles/liter.

Other available solvents were qualitatively compared with acetone by observing the intensity of the color imparted to the solution and the amount of undissolved complex left after exposing a sample to the solvent for a given time. The solubilities of rubrocurcumin in the solvents tested fall into three distinct classifications, as shown in Table I.

The solvents which develope little if any color on exposure to rubrocurcumin are listed in Table I in the insoluble section. Those which become dark red or purple but which cause little or no visible decrease in the quantity of complex are classified slightly soluble. Acetone is placed in this classification. The only solvents tested in which rubrocurcumin is appreciably soluble are dimethylformamide and dimethylsulfoxide, both of which have very low evaporation rates.

TABLE I

Rubrocurcumin Solubility Studies

I.   Insoluble

      A. Hexane
      B. Cyclohexane
      C. Benzene
      D. Toluene
      E. Ether
      F. Carbon tetrachloride
      G. Tetrachloroethylene
      H. Water

II.   Slightly Soluble

      A. Acetone
      B. Methyl ethyl ketone
      C. Methyl alcohol
      D. Ethyl alcohol
      E. n - Propyl alcohol
      F. iso - Propyl alcohol
      G. Ethylene glycol
      H. Ethylene glycol mono methyl ether
      I. Ethylene glycol mono ethyl ether
      J. Methyl acetate
      K. Ethyl acetate
      L. Chloroform
      M. Dioxane
      N. Tetrahydrofuran

III.   Soluble

      A. Dimethylformamide
      B. Dimethylsulfoxide

B. Fluorescence of Rubrocurcumin

While conducting the solubility studies, it was
observed that rubrocurcumin exhibits a strong pink
fluorescence in chloroform. A search of the literature
showed that Ghosh (7) had reported this fluorescence
in 1919, however no one has developed a quantitative
method for boron based on this phenomenon. An absorption
and fluorescence spectrum of rubrocurcumin in chloroform,
run on an Aminco Kiers Spectrophosphorimeter, is presented
as Figure I.

The feasibility of applying this fluorescence to the
determination of boron was briefly investigated, however
it was decided that such an investigation was beyond the
scope of the proposed work. Figure II is an example of a
standard curve obtained by plotting μg boron / 25 ml
against fluorescence using a Turner Fluorometer. The
samples were prepared by the modified Naftel procedure
as described by Pollio (15) except that chloroform was
used to dissolve the evaporated sample and that the
readout was fluorometric instead of colorimetric.

Figure I

The Absorption and Fluorescence Spectrum of

Rubrocurcumin in Chloroform

An Aminco - Kiers Spectrophosphorimeter
was used to obtain this spectrum.

_____ Absorption

------------- Fluorescence

mμ →
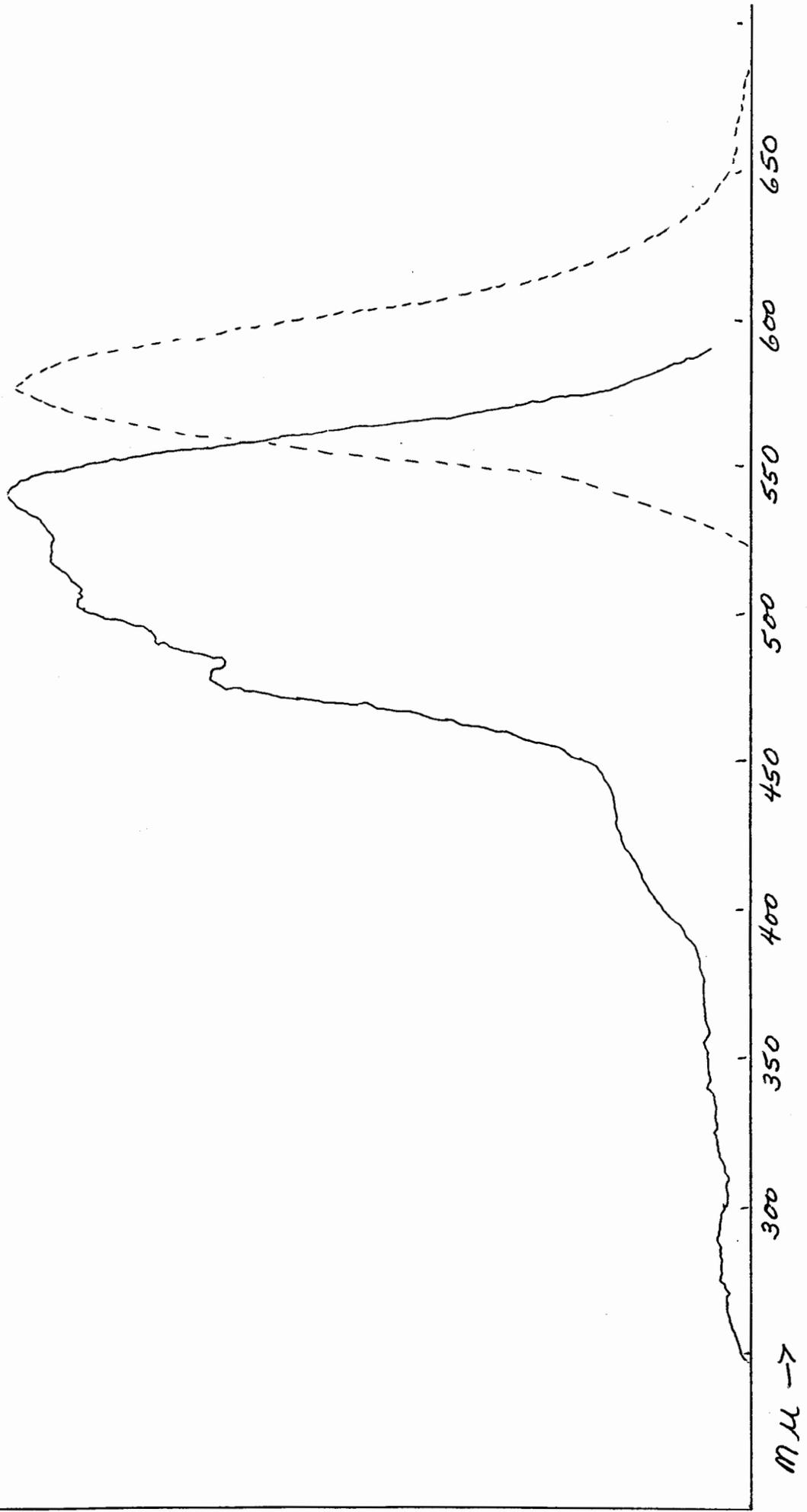
Figure II

Calibration Curve For Fluorometric Determination

of Boron in Chloroform
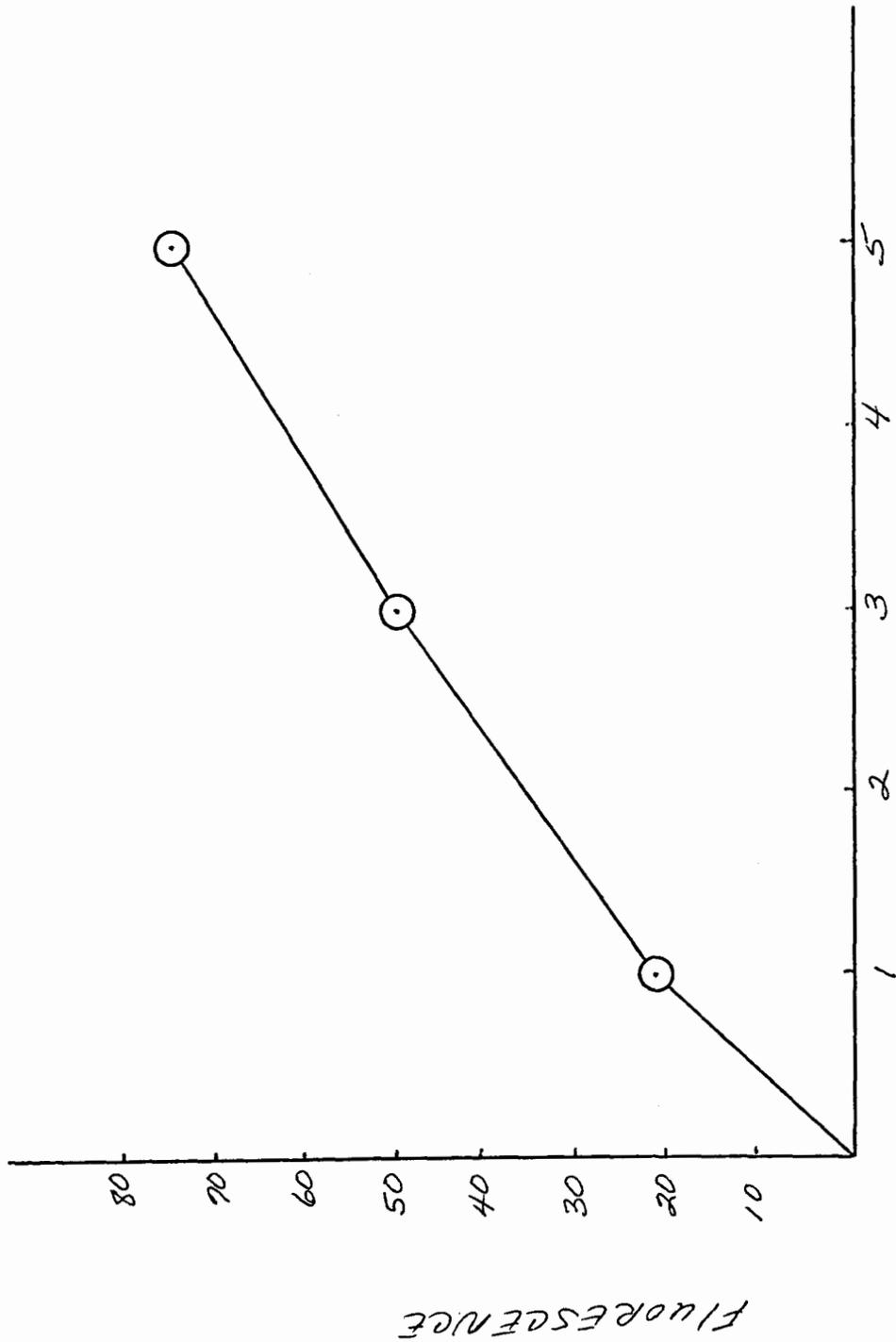
CONCENTRATION - μg BORON / 25 ml

FLUORESCENCE

V. Molecular Weight of Rubrocurcumin

It was learned from the solubility investigations
above that the only solvents in which rubrocurcumin is
sufficiently soluble to give reproducible readings in the
Vapor Pressure Osmometer are dimethylformamide and
dimethylsulfoxide. Both of these solvents, however,
have such low evaporation rates that reproducible $\triangle R$
readings cannot be obtained in a reasonable time if they
are used in the osmometer. Therefore it was apparent
that in order to use the osmometer to determine the
molecular weight of rubrocurcumin, its sensitivity would
have to be increased.

A significant sensitivity increase was obtained by
modifying the manner in which the sample was applied to
the thermistor bead. If one solvent drop is left on the
reference bead for the entire set of readings, as the
procedure calls for, the solvent drop is in equilibrium
with its surroundings. A sample drop placed on the measur-
ing bead requires about 10-15 minutes to equilibrate.
With most solvents, however, the $\triangle R$ reading reaches about
3/4 of the equilibrium value and is reproducible in about
two minutes. The $\triangle R$ reading is usually made two minutes
after placing the sample drop on the measuring thermistor.

It was observed that if drops are placed on both beads at approximately the same time, this same equilibrium difference is obtained in about thirty seconds. Figure III is a comparison of the sensitivity of the two methods discussed above for applying the drops to the thermistor beads.

This method of "simultaneous" addition was used for all of the work reported in this paper. A solvent drop was placed on the reference bead first and as quickly as possible (usually within 10-15 seconds) a drop of solution was placed on the measuring bead. The $\triangle R$ reading was made two minutes later if acetone was the solvent and eight minutes later for dimethylformamide.

A set of samples was prepared by allowing rubrocurcumin to stand for several hours in a beaker with acetone. The acetone solutions were then decanted, samples were read on the osmometer and aliquots were evaporated in tared porcelain dishes in order to determine the weight concentration. The solubility of the complex in acetone is so low, however, that the readings were low and not very reproducible. It was felt that by making ten $\triangle R$ readings on each sample, the average would be close to the true value even though there was a large variation between individual readings.

The first five samples (all from the same batch of rubrocurcumin) gave low molecular weights. This sample

Figure III

Comparison of Methods of Applying Solvent Drops to

The Reference Bead of The Vapor Pressure Osmometer

$\triangle$ = One drop left on reference bead for
  entire set of readings

$\odot$ = Drop placed on reference bead just
  before sample drop is placed on sample bead

(Readings made two minutes after sample –
  Benzil in acetone – was applyed to meas-
  uring bead)
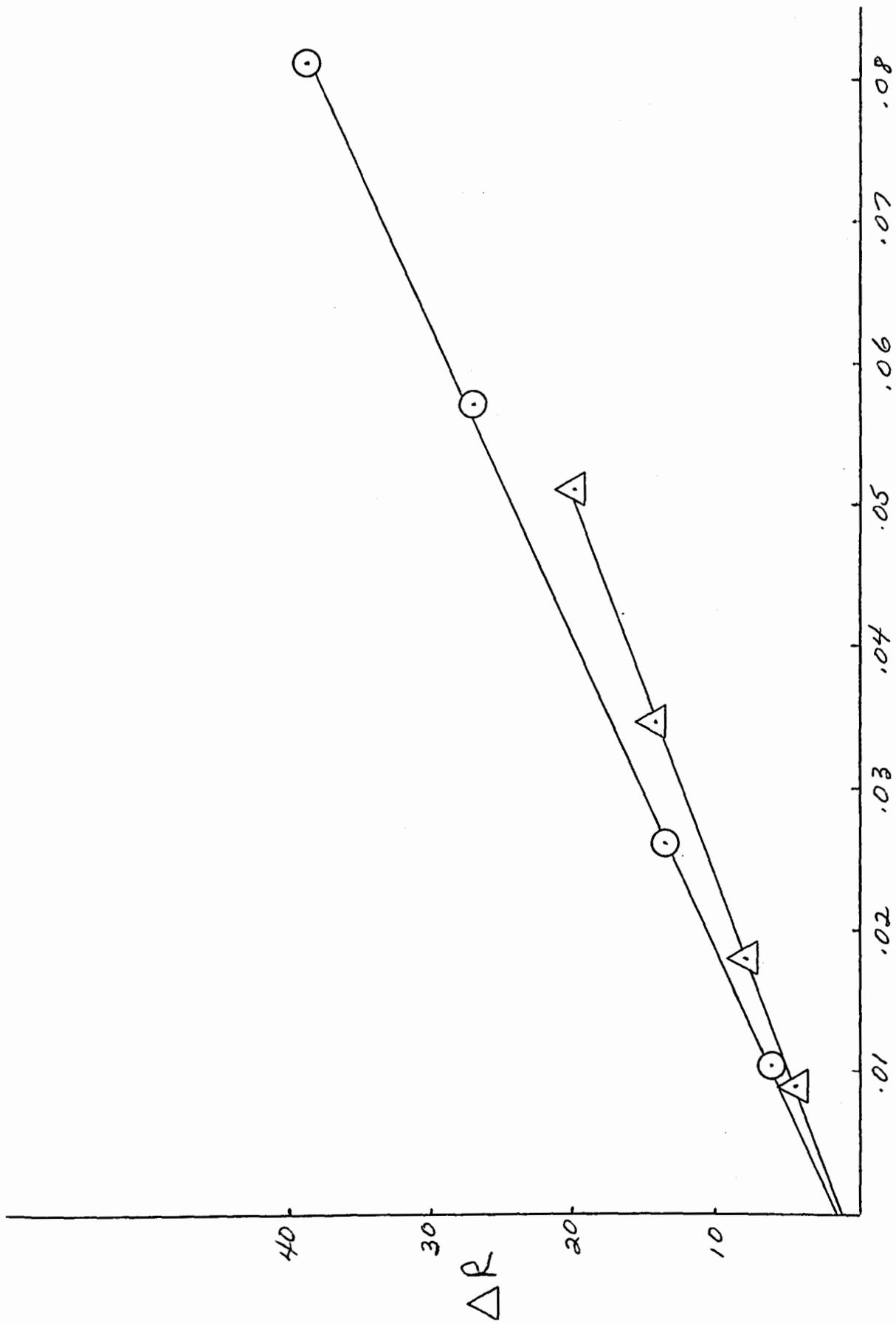
CONCENTRATION - Moles / Liter

of rubrocurcumin was apparently contaminated with low molecular weight impurities. This experiment was repeated using freshly prepared and carefully purified rubrocurcumin and the average of the molecular weights obtained was less than 3% higher than the calculated value for a 1:1:1 complex. In order to test the method to see if it would give reliable results, four samples of recrystallized curcumin, in the same concentration range, were determined by this procedure. The results of this experiment are given in Table II.

The average molecular weight of rubrocurcumin as determined by this procedure is 481 with a relative standard deviation of $\pm$ 3%. The average of the relative standard deviations of the ΔR readings made on each sample is $\pm$ 16%.

Table II

The Molecular Weight of Rubrocurcumin

Wt. Conc. g/1	$\triangle R$ (Ave. of 10)	Molar Conc. m/1	M. Wt.
0.236	0.33	.00050	471
0.524	0.74	.00112	467
0.246	0.32	.00049	502
0.344	0.47	.00072	480
		Ave.	481

The Molecular Weight of Recrystallized Curcumin

(Actual Value = 368.4)

Wt. Conc. g/1	$\triangle R$ (Ave. of 10)	Molar Conc. m/1	M. Wt.
0.300	0.39	.00082	368
0.867	1.36	.00021	413
0.664	1.20	.00018	365
0.199	0.36	.00055	333
		Ave.	370

Next, a mixture of acetone and dimethylsulfoxide (DMSO) was tested hoping to combine the favorable volatility of acetone and the favorable solubility of the complex in DMSO. The instrument was balanced as usual with 2:1 acetone: DMSO on both thermistor beads. When standards or samples dissolved in this mixture were tested, the reading was always zero - indicating that the mixture was acting as a solution of DMSO in acetone and that the sample molecules were so strongly associated with the DMSO molecules that their presence in the solution did not increase the total number of molecules present.

As was shown above, the solubility of rubrocurcumin in all of the volatile solvents tested is too low to yield solutions sufficiently concentrated to give reliable readings with the VPO. Therefore, since the instrument operates on a colligative principle and measures the total number of moles present regardless of their chemical nature, it was decided to add carefully weighed amounts of benzil to solutions of rubrocurcumin in acetone in order to raise the total molar concentration of the solutions above 0.025 moles/liter. The VPO is capable of accurately measuring differences in concentration of the order of 0.0001 moles/liter in this range. It was then possible to determine the molar concentration of rubrocurcumin in the solution by subtracting the concentration of added benzil

from the total molar concentration measured with the VPO.

The complex was allowed to stand for several hours in an open beaker of acetone in order to give the complex time to dissolve and to allow some of the acetone to evaporate - further concentrating the solution. At the same time, a beaker of acetone was left open to the atmosphere so that it would be conditioned in the same way as the samples. Since the concentration of rubrocurcumin was low, it was important that the solvent placed on the reference bead and used for preparation of standards had the same concentration of impurities. Meanwhile sufficient benzil was weighed into tared volumetric flasks to make the concentration in the flasks between .025 and .035 moles/liter when diluted to volume. The weight concentration of rubrocurcumin in the acetone solution was determined as before and then the volumetric flasks were filled to the mark with this solution. The benzil - rubrocurcumin - acetone solutions were read on the osmometer along with a set of four standards in the .025 - .035 molar range. The $\triangle$R readings for the samples were multiplied by the factor $\dfrac{moles/liter}{\triangle R}$ calculated

from the readings obtained with the standards. The molecular weight was calculated by the following equation:

$$\text{Molecular Weight} = \frac{\text{g/l of rubrocurcumin}}{\text{total m/l} - \text{m/l of benzil}}$$

The molecular weight of recrystallized curcumin was also determined by this procedure. The results of these determinations are given in Table III.

The average molecular weight of rubrocurcumin as determined by this procedure is 455 with a relative standard deviation of $\pm$ 7%. The average of the relative standard deviations for the Δ R readings made on each sample is $\pm$ 3%.

Table III

The Molecular Weight of Rubrocurcumin in a Solution of

Benzil in Acetone.

Conc. of Benzil m/l	$\triangle R$ (Ave. of 5)	Total Conc. m/l	Excess Conc. m/l	Wt. Conc. of Complex g/l	M. Wt.
.03287	14.60	.03416	.00129	.549	426
.02797	12.45	.02913	.00116	.549	473
.03210	13.22	.03093	.00117	.549	470
.02635	11.74	.02747	.00112	.549	490
.03120	13.88	.03248	.00128	.549	429
.02797	12.43	.02909	.00112	.549	490
.03125	13.90	.03253	.00128	.549	429
.02973	13.22	.03093	.00120	.549	458
.02764	12.29	.02876	.00112	.549	490
.03558	15.81	.03700	.00142	.549	387
.03392	13.11	.03536	.00144	.549	381
.03140	14.80	.03210	.00070	.344	491
.02792	12.11	.02847	.00055	.252	458
.02916	12.63	.02969	.00053	.252	476
.03173	13.73	.03228	.00055	.252	458
.03391	14.65	.03444	.00053	.252	<u>476</u>

Ave. 455

Molecular Weight of Recrystallized Curcumin in Solutions of

Benzil in Acetone.

Conc. of Benzil m/l	$\triangle R$ (Ave. of 5)	Total Conc. m/l	Excess Conc. m/l	Wt. Conc. of Complex g/l	M. Wt.
.02703	12.08	.02823	.00120	.456	380
.02635	11.80	.02760	.00125	.456	365
.02939	13.10	.03062	.00123	.456	<u>372</u>

Ave. 372

Preliminary attempts to use dimethylformamide (DMF) as a solvent in the osmometer showed that the readings would not reach equilibrium in a reasonable time due to its low rate of evaporation. Readings which were made one hour after depositing the drops were still not reproducible. However, if a thin film of liquid was placed on the thermistor beads by barely touching the beads with the syringes, reproducible readings could be made in eight minutes. The sensitivity of the instrument when DMF is used is about 1/10 that when acetone is used. Figure IV is a comparison of standard curves run with benzil dissolved in acetone and DMF.

The data obtained using DMF as solvent on 16 samples of rubrocurcumin and three samples of recrystallized curcumin are given in Table IV.

The average molecular weight of rubrocurcumin as determined by this procedure is 462 with a relative standard deviation of $\pm$ 3%. The average of the relative standard deviations for the ΔR readings made on each sample is $\pm$ 6%.

Table IV

Molecular Weight of Rubrocurcumin

(Solvent = DMF)

Wt. Conc. g/l	△R (Ave. of 5)	Molar Conc. m/l	M. Wt.
3.60	0.85	.0085	430
5.12	1.20	.0118	434
6.44	1.27	.0139	464
4.20	0.87	.0088	477
5.06	1.00	.0106	477
3.98	0.86	.0086	463
5.74	1.22	.0125	459
4.79	0.95	.0102	470
4.40	0.93	.0098	449
4.60	0.94	.0099	465
6.15	1.16	.0130	473
5.85	1.14	.0127	461
5.76	1.08	.0120	480
4.64	0.94	.0100	464
5.66	1.10	.0123	460

Ave. 462

Molecular Weight of Recrystallized Curcumin

(Solvent = DMF)

Wt. Conc. g/l	△R (Ave. of 5)	Molar Conc. m/l	M. Wt.
9.48	2.15	.0352	376
5.37	1.27	.0147	365
6.49	1.48	.0175	370

Ave. 370

Figure IV

Comparison of Acetone and DMF as Solvent for

Molecular Weight Determinations With The

Vapor Pressure Osmometer

⊙ Benzil in Acetone - reading made two
minutes after applying sample drop

△ Benzil in DMF - reading made eight
minutes after applying sample drop
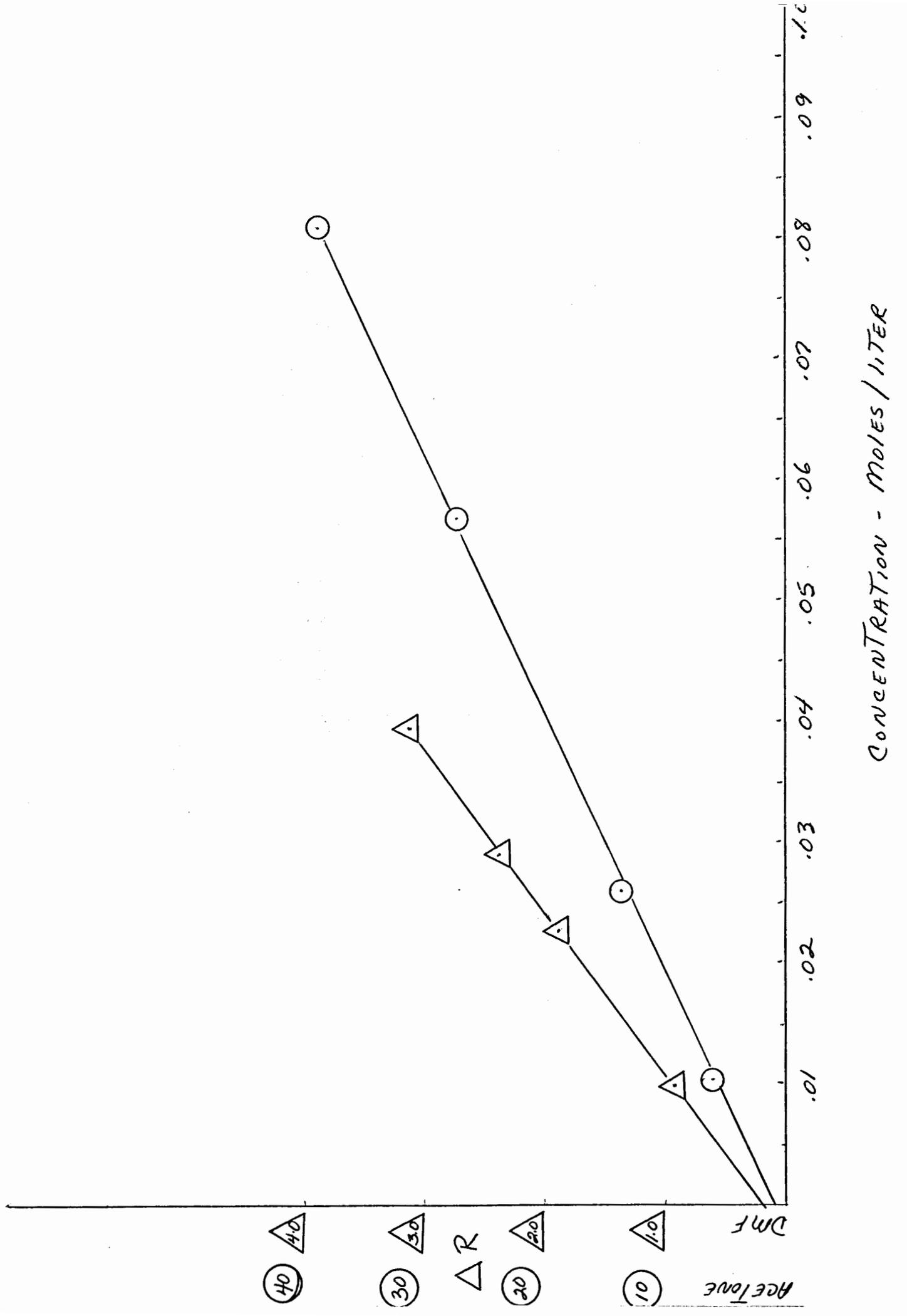
ConcenTration - Moles / liter

VII.   Determination of The Molecular Weight of Rosocyanin

As mentioned earlier in this paper, there has been a
great deal of discussion concerning the structure of
rosocyanin for several years.   Therefore it was decided to
obtain the molecular weight of this compound in order to
determine whether it is a 1:1 or 2:1 curcumin:boron
complex.   Since the solubility properties of rosocyanin
are very similar to those of rubrocurcumin, the molecular
weight determinations were made using DMF as a solvent.
The procedure used was the same as that described above
for the determination of the molecular weight of rubrocurcumin
in DMF.

The data summarized in Table V indicates that rosocyanin
forms a 1:1 complex under the conditions which existed.
The calculated molecular weight for a 1:1 complex is 378.4.

Table V

Molecular Weight of Rosocyanin

(Solvent = DMF)

Wt. Conc. g/l	$\triangle$ R (Ave. of 5)	Molar Conc. m/l	Mol. Wt.
4.35	1.04	.0120	362
3.89	0.89	.0103	378
4.52	1.01	.0116	389
3.07	0.72	.0083	370
		Ave.	374

VIII. Determination of Molar Ratio of Boron
to Curcumin in Rosocyanin

The molar ratio of rosocyanin was determined using
the Vapor Pressure Osmometer to measure the total molar
concentration of a DMF solution of the residue after a solu-
tion containing boric acid and curcumin was allowed to
evaporate to dryness. For example, in one test an acetone
solution of .00107 moles of boric acid and .00221 moles of
curcumin was allowed to evaporate to dryness and the residue
was dissolved and washed into a 100 ml volumetric flask
with DMF. If no reaction had occured, the total molar
concentration of the resulting solution would be .0107
moles/liter of boric acid + .0221 m/l of curcumin or .0328
m/l. If a 1:1 complex formed, there would be .0107 m/l
of rosocyanin plus .0221 - .0107 or .0114 m/l of excess
curcumin for a total concentration of .0221 m/l. In the
event of a 2:1 complexation the concentration would be .0107
m/l of 2:1 complex plus .0221 - .0214 or .0007 m/l of excess
curcumin for a total molar concentration of .0114.

The results given in Table VI show that rosocyanin
consists essentially of a 1:1 curcumin:boron complex. All
of the values are slightly lower than expected for a 1:1
complex, however, indicating that a small amount (about 2%)

of 2:1 complex might be formed. In these experiments the
molar quantity of curcumin used was twice that of boric
acid. Under different conditions the extent of formation
of 2:1 complex might be greatly altered.

Table VI

Ratio of Boron:Curcumin in Rosocyanin

(Solvent = DMF)

Boric Acid m/l	Curcumin m/l	Conc. if 1:1 reaction occurs m/l	$\triangle R$ (Ave. of 5)	Conc. m/l
.0107	.0221	.0221	1.83	.0216
.0110	.0224	.0224	1.87	.0220
.0112	.0228	.0228	1.85	.0219
.0116	.0229	.0229	1.89	.0223

IX. Determination of the Boron - Oxalic Acid Ratio

According to the mechanism of the boron - oxalic acid -
curcumin reaction proposed by Powell, Hardcastle and
Poindexter (16), oxalic and boric acids react first to form
a 1:1 complex as the solution evaporates, making it easier
for the curcumin to enter into the complex.

In order to prove that such a boron - oxalic acid
reaction does occur, the same procedure used above for the
determination of the molar ratio of rosocyanin was followed.
Acetone rather than DMF was used to dissolve the residue
because of its higher sensitivity.

The results of five determinations summarized in Table VII clearly show that boric acid and oxalic acid form a 1:1 complex when a solution of the two evaporates to dryness.

The residues from the first two samples were dissolved in 50 ml of acetone and apparently this was not enough solvent to thoroughly wash the samples into the volumetric flasks. This probably explains the low value obtained for the first sample. For the next three samples, the amounts of boric acid and oxalic acid were doubled so that 100 ml of acetone could be used to wash the beaker.

The higher than predicted values indicate that the reaction is not quite complete.

Table VII

Molar Ratio of The Oxalic Acid - Boric Acid Complex

Boric Acid m/1	Oxalic Acid m/1	Conc. if 1:1 reaction occurs m/1	$\triangle$ R (Ave. of 5)	Conc. m/1
.0248	.0502	.0502	17.10	.0405
.0248	.0502	.0502	22.90	.0540
.0278	.0506	.0506	21.10	.0502
.0274	.0511	.0511	22.16	.0525
.0260	.0509	.0509	22.33	.0530

SUMMARY

In this research project, information was sought which could be used to determine the structures of rubrocurcumin (the compound formed by the reaction of boron, oxalic acid and curcumin) and rosocyanin (the compound formed by the reaction of boron with curcumin in the absence of oxalic acid) and to help us to better understand the mechanism proposed by Powell, Hardcastle and Poindexter (16) for the formation of rubrocurcumin in the Naftel procedure.

The first successful determination of the molecular weight of rubrocurcumin was made. The average of the rubrocurcumin molecular weight data reported in this paper is 461 - only 1.5% lower than the calculated molecular weight of 468.5 for a 1:1:1 complex.

In order to test the methods used, the molecular weight of recrystallized curcumin was determined by the same methods used to determine the molecular weight of rubrocurcumin. The average of the curcumin molecular weight determinations is 371 - less than 1% higher than the accepted value of 368.4.

The molecular weight and molar ratio of the components

SUMMARY

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In order to test the methods used, the molecular weight of recrystallized curcumin was determined by the same methods used to determine the molecular weight of rubrocurcumin. The average of the curcumin molecular weight determinations is 371 - less than 1% higher than the accepted value of 368.4.

The molecular weight and molar ratio of the components

of rosocyanin were determined showing that the rosocyanin molecule consists of one molecule of boron complexed with one molecule of curcumin. A molecular weight of 374 was obtained which is about 1% lower than the calculated molecular weight of 378.4. There was some evidence that a small amount of 2:1 curcumin:boron complex might have formed also and it is conceded that under different conditions the amount of 2:1 complex formed might be even higher.

Powell and co-workers proposed that a 1:1 boron:oxalic acid complex formation is the initial and sensitizing step in the formation of rubrocurcumin in the Naftel procedure. Investigations of the total molar concentrations of solutions obtained by dissolving the residues left on evaporation of solutions containing boric and oxalic acids to dryness prove that such a 1:1 reaction does occur.

In summary, all of the data reported in this thesis tends to support the Powell, Hardcastle and Poindexter mechanism for the formation of rubrocurcumin.

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