

THE MEASUREMENT OF THE EXTINCTION COEFFICIENT OF A FISH LIVER OIL

Thesis for the Degree of M. S. MICHIGAN STATE COLLEGE William Henry Martin 1942



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by

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Department of Chemistry

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Appreciation

The writer wishes to express his appreciation to Dr. D. T. Ewing for his helpful suggestions and guidance during the course of this investigation. Introduction: When Ewing, Vandenbelt, Emmett, and Bird (2) evaluated fish liver oil #2898 using a sector photometer they reported a deviation of three and one half percent in their results. Escheile (12), using a photoelectric spectrograph on a similar experiment reduced the deviation to two tenths of one percent.

The investigation reported in this paper is an attempt to discover a method of using the sector photometer but at the same time reducing the deviation from the three and one half percent reported by Ewing <u>et al</u>. using the <u>sector</u> instrument.

One limitation of the sector photometer is the inability to photograph successive absorption spectra closer than one half unit on the adjustable sector scale. A second limitation results from errors introduced by pipetting --- drainage, density changes of the solvent, and fluctuation of pipette volume. The inaccuracy of visual interpretation as compared to that of the photoelectric cell presents a third limitation.

The apparatus used in this study was a Bausch and Lomb ultraviolet sector photometer equipped with a Bausch and Lomb medium quarts optical system. The continuum is that of a Hilger hydrogen tube operating at a potential of 2,000 volts. The one centimeter glass cells with quartz ends are housed in metal cases. A Bausch and Lomb density comparator was used for measuring the

the densities of the photographic spectra.

The materials used were Eastman practical grade of hexane for solvent; Dastman Spectrum Analysis "1, Antihelation photographic plates; photographic developer D-19. The substance used was fish oil #2898 supplied by Parke Davis Company of Detroit, Michigan.

Experimental: From a hydrogen discharge tube two beams of ultra violet radiation are passed through the spectrophotometer. The top beam $--I_0$ --- passes through the solution and then the "loo percent" sector. The two beams are then collimated, passed through a medium quartz cornu prism and are adjacently photographed upon the plate.

A series of exposures is made differing only in the ratio of the log of I_0/I . The quantity of light passing through the solvent is kept a constant --- that is the product of the exposure time and the sector setting is equal to a constant.

The concentration of the solution is adjusted in such a manner that the maximum extinction occurs in the region of a sector setting of ten. All concentrations expressed are in terms of grams of solute per 100 cubic centimeters of solvent. Laboratory experience has demconstrated that in the region of a sector setting of ten the accuracy of this instrument is at a maximum.

* See page 17

The plate is then developed in D-19 developer for two minutes, fixed in hypo for two minutes, and washed in running water fifteen minutes before drying.

The extinction point of an absorption spectrum is the point where the densities of the I_0 beam and the I beam are equal. The maximum extinction point is the extinction point occurring on the band of the lowest sector setting.

The relative densities of the I_0 and I beams are measured on a Eausch and Lomb density comparator. The ratio of deflections of the I_0 and I beams on the microphotometer is proportional to the ratio of the relative densities of these beams. The ratio of these deflections, I_0/I for a series of bands is plotted against the corresponding sector settings.* For bands of lower sector settings than the maximum extinction point, the ratio will be greater than unity; for bands of higher sector settings than the maximum extinction point, the ratio will be less than one. The point where the curve crosses the unity line is considered the isodensity point and its corresponding sector setting is read on the "Y" axis.

In making any absorption determinations on the sector photometer, it is imperative that the instrument be in proper alignment.

* See page 4



The first step in aligning the instrument is to open the slit, remove the plate holder, and illuminate the source. The image of the source, two dark circular lines, may be seen by viewing the spectrum. By moving the remote end of the sector section of the photometer laterally, these images may be brought into the center of the spectrum as defined by the diaphrasm. The slit is then closed to the proper width and the relative intensities of the two beams which make up the band may be investigated. When the band is photographed through open sectors, with no cells in the instrument, the ratio of the microphotometer deflections from the bound should be one if the densities are equal. In the event that this condition is not realized, the densities of these beams may be changed by adjusting the alignment of the discharge tube. When the tube end which is more remote from the slit is raised, the "I" beam will become darker and vice versa. If the two beams tend to overlap at the center, thus forming a dark line through the center of the band, the following adjustment of the instrument may be made to correct this defect in alignment. If the distance between the Fresnel biprism and the slit is diminished, the overlapping of the lines lessens. Buach and lomb recommend that the biprism be placed as near the slit as possible without damage to either the slit or the birrism. However on the instrument used, it was

impossible to place the biprism as close as desired. A minimum distance was used.

A series of determinations were made using fish oil $\frac{2}{2}$ of greviously reported by Ewing, Vandenbelt, Emmett, and Bird (2). The plates were visually spotted by two individuals who had had previous experience in spotting absorption spectra. The maximum extinction values were then calculated according to Beer's relationship:

$$E_{lcm}^{1,2} = \frac{log I_c/I}{c a}$$

I is equal to the incident light (160 percent).
I equals the percent of light transmitted at the wave length of the absorption maximum.
c equals the percent concentration of the solution.
d is equal to the length of the cell in centimeters.

Table A indicates that the results of visual spotting may deviate as much as 2.72 percent within themselves. Also it should be noted that when the mean values of one reader are compared with those of another, there is a difference of only .25 percent.

The volumes used in table A in determining the percent concentration were pipetted and then carefully weighed. By using the density of hexane at the temperature when the runs were made, its correct volume may be determined. From this the correct values of the concentrations may be calculated. Table B shows this concentration correction applied to the sector settings of table A.

Flate	% Conc.	ISector	II Sector	I F.Lom	II El% lcm
107	.02260	10.00	10.00	20.67	60.67
168	.01225	10.50	10.00	20.15	51.00
169	-05275	10.11	9.50	20.50	61. 21
170 1793	02020	9.50 10 EO		20.87	
172B	-02220	10.25	10.50	50.45	
173à	.02270	10.00	10.00	20.58	20.58
172B	.03270	10.00	10.00	20.58	20.58
174A	.02225	9.25	9.50	22.09	51.69
174B	.03225	10.00	9.50	21.00	21.69
	······································	I		II	
Range		1.75		1.50	
Deviat	ion	•£7		•75	
% Voon V		2.72			
Lean v % Diff	^e erence			00.54	
of Mea	in Values		• 25		
	9-1- 1 -1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1	23.07.01.0.00.003.0			
m · m · m	D 4 000 -		• • • • • • • • • • • • • • • • • • •		
TABLE	B A COLF #2898 AND WE	ARISON OF AT 520 NU IGHT-DENS	MAXIMUM EX AS DETERNI ITY RUFINER	TINCTION NED BY VI ENTS	VALUES OF ISUAL METHOD
TABLE Plate	B A COLF #2898 AND WE % Conc. (Correcte	ARISON OF AT 520 LU IGHT-DENS I Sect d)	MAXIMUM EX AS DETERNI ITY REFINER or 11 Sect	TINCTION NED BY VI ENTS or I Elg	WALUES OF ISUAL METHUD
TABLE Plate 167	B A COLF #2898 AND WE % Conc. (Correcte .C2285	ARISON OF AT 520 NU IGHT-DENS I Sect d) 10.00	MAXIMUM EX AS DETERNI ITY REFINED or II Sect 10.00	TINCTION NED BY VI ENTS for I E	WALUES OF ISUAL METHOD m II El% 5 20.45
TABLE Plate 167 168	B A COLF #2898 AND WE % Conc. (Correcte .C2285 .02225	ARISON OF AT 520 NU IGHT-DENS I Sect d) 10.00 10.50	MAXIMUM EX AS DETERNI ITY REFINER or II Sect 10.00 10.00	ETINCTION INED BY VI ENTS For I E12 20.45 20.45	WALUES OF ISUAL METHOD m II El% m So.45 5 20.45
TABLE Plate 167 168 169	B A COLF #2898 AND WE % Conc. (Correcte .C2285 .02225 .02210 .02265	ARISON OF AT 520 MU IGHT-DENS I Sect d) 10.00 10.50 10.00 0.50	MAXIMUM EX AS DETERNI ITY REFINER or II Sect 10.00 10.00 9.50	TINCTION NED BY VI ENTS for I $E_{10}^{1/2}$ 20.45 20.25 20.20	HALUES OF ISUAL METHOD II E1% II E1% II 0.45 II 0.45 II 0.65 II 0.65
TABLE Plate 167 168 169 170 1724	B A COMF #2898 AND WE % Conc. (Correcte .C2285 .02210 .02285 .02210	ARISON OF AT 520 NU IGHT-DENS I Sect d) 10.00 10.50 10.00 9.50	MAXIMUM EX AS DETERNI ITY REFINER or II Sect 10.00 10.00 9.50 10.00	ETINCTION $ENTS$ $ENTS$ $EO.4E$ $EO.4E$ $EO.2E$ $EO.2C$ $EO.2C$ $EO.2C$	HALUES OF ISUAL METHUD II E 5 20.45 5 20.45 5 20.45 5 20.45 5 20.25 20.85 20.55
TABLE Plate 167 168 169 170 172A 172B	B A COLF #2898 AND WE % Conc. (Correcte .C2285 .02285 .02285 .02285 .02285 .02285	ARISON OF AT 520 NU IGHT-DENS I Sect d) 10.00 10.50 10.00 9.50 10.50 10.25	MAXIMUM EX AS DETERNI ITY REFINER or II Sect 10.00 10.00 9.50 10.00 10.00 10.50	$\begin{array}{c} \text{TINCTION} \\ \text{INED BY VI} \\ \text{ENTS} \\ \hline \\ \text{Sor I E}_{10}^{12} \\ \text{Sor 20.20} \\ \text{SO.20} \\$	WALUES OF ISUAL METHOD II E1% II E1% Im II E1%
TABLE Plate 167 168 169 170 172A 172B 172A	B A COMF #2898 AND WE % Conc. (Correcte .C2285 .C2210 .C2285 .C2255 .C2255 .C2255 .C2255 .C2270	ARISON OF AT 520 LU IGHT-DENS I Sect d) 10.00 10.50 10.00 9.50 10.50 10.50 10.50 10.50 10.50	MAXIMUM EX AS DETERNI ITY REFINER or II Sect 10.00 10.00 9.50 10.00 10.00 10.00	ETINCTION INED BY VI ENTS for I E17 20.45 20.25 20.20 20.20 20.20 20.40 20.40	HALUES OF ISUAL METHOD $\frac{11 \text{ E}_{1\text{cm}}^{1\%}}{11 \text{ E}_{1\text{cm}}^{1\%}}$ 5 20.45 5 20.45 5 20.45 5 20.65 29.55 5 20.70 10 05 20.60
TABLE Plate 167 168 169 170 172A 172A 172A 172A	B A COLF #2898 AND WE % Conc. (Correcte .C2285 .C221C .C2285 .C2255 .C2255 .C2255 .C2255 .C2255 .C2270 .02270	ARISON OF AT 520 NU IGHT-DENS I Sect d) 10.00 10.50 10.00 9.50 10.25 10.00 10.00	MAXIMUM EX AS DETERNI ITY REFINER or II Sect 10.00 10.00 10.00 10.00 10.00 10.00 10.00	$\begin{array}{c} \text{TINCTION} \\ \text{NED BY VI} \\ \text{ENTS} \\ \hline \\ \text{for I E}_{10}^{12} \\ \text{Core } \\ \ \ \ \text{Core } \\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \$	WAIUES OF ISUAL METHUD II E1% II E1% 5 6 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 <
TABLE Plate 167 168 169 170 1728 1728 1728 1728 1728	B A COLF #2898 AND WE % Conc. (Correcte .C2285 .02255 .02255 .02255 .02255 .02255 .02255 .02255 .02270 .02270 .02270 .02250	ARISON OF AT 520 NU IGHT-DENS I Sect d) 10.00 10.50 10.50 10.50 10.50 10.50 10.50 10.50 10.50 10.50 10.25 10.00 9.25	MAXIMUM EX AS DEFTER T ITY REFINER or II Sect 10.00 10.00 9.50 10.00 10.00 10.00 10.00 10.00 9.30	$\begin{array}{c} \text{TINCTION} \\ \text{INED BY VI} \\ \text{ENTS} \\ \hline \\ \text{Sor I E}_{10}^{12} \\ \hline \\ \text{Sor I C}_{10}^{12} \\ \hline \\ \ \\ \ \\ \text{Sor I C}_{10}^{12} \\ \hline \\ \ \\ \ \\ \ \\ \ \\ \ \\ \ \\ \ \\ \ \\ \$	HALUES OF ISUAL METHOD II E1% II E1% 5 20.45 5 20.45 5 20.45 5 20.45 5 20.65 29.55 5 20.60 20.60 20.60 21.50
TABLE Plate 167 168 169 170 172A 172B 172A 172B 172A 174B	B A COLF #2898 AND WE % Conc. (Correcte .C2285 .02255 .02255 .02285 .02285 .02255 .02255 .02255 .02255 .02270 .02250 .02250	ARISON OF AT 520 NU IGHT-DENS I Sect d) 10.00 10.50 10.00 9.50 10.50 10.50 10.00 9.25 10.00	MAXIMUM EX AS DETERNI ITY REFINER or II Sect 10.00 10.00 10.00 10.00 10.00 10.00 10.00 10.00 9.50 9.50 9.50	$\begin{array}{c} \text{TINCTION} \\ \text{INED BY VI} \\ \text{ENTS} \\ \hline \\ \text{Sor I E}_{10}^{12} \\ \text{Sor I C}_{20}^{12} \\ \text{Sor I C}_{20}^{$	HALUES OF ISUAL METHOD 20.45 20.45 20.45 20.85 20.85 20.55 20.65 20.65 20.60 20.60 20.60 20.60 20.60 20.45 20.60 20.60 20.60 21.40
TABLE Plate 167 168 169 170 1728 1728 1728 1728 174A 1748	B A COLF #2898 AND WE % Conc. (Correcte .C2285 .C2255 .C2255 .C2255 .C2255 .C2255 .C2255 .C2255 .C2255 .C2270 .C2250 .C2250	ARISON OF AT 520 NU IGHT-DENS I Sect d) 10.00 10.50 10.00 9.50 10.50 10.50 10.50 10.00 9.25 10.00 9.25 10.00	MAXIMUM EX AS DEFTER T ITY REFINER or II Sect 10.00 10.00 9.50 10.00 10.00 10.00 10.00 9.50 9.50 9.50	TINCTION NED BY VI ENTS For I E17 20.45 20.25 20.20 20.20 20.20 20.60 20.60 20.60 20.60 20.60 20.60 20.60 20.60 20.60 20.75	HALUES OF ISUAL METHOD II E1% <
TABLE Plate 167 168 169 170 172A 172B 172A 172B 174A 174B Range Devict	B A COMF #2898 AND WE % Conc. (Correcte .C2285 .C2255 .C2255 .C2255 .C2255 .C2255 .C2255 .C2255 .C2255 .C2270 .02250 .02250	ARISON OF AT 520 MU IGHT-DENS I Sect d) 10.00 10.50 10.00 9.50 10.00 9.50 10.00 9.25 10.00 9.25 10.00 10.00 9.25 10.00	MAXIMUM EX AS DETERNI ITY REFINER or II Sect 10.00 10.00 10.00 10.00 10.00 10.00 10.00 9.50 9.50 9.50	$ \begin{array}{r} TINCTION \\ INED BY VI \\ ENTS \\ \hline I El% \\ Sor I El% \\ 20.48 \\ 20.28 \\ 20.20$	HALUES OF ISUAL METHOD $\frac{11}{2}$ E $\frac{11}{2}$ E
TABLE Plate Plate 167 168 169 170 172A 172B 172A 172B 172A 174B 174A 174B Range Deviat % "	B A COLF #2898 AND WE % Conc. (Correcte .C2285 .02255 .02255 .02255 .02270 .02270 .02250 .02250 .02250	ARISON OF AT 520 NU IGHT-DENS I Sect d) 10.00 10.50 10.50 10.50 10.50 10.50 10.00 9.25 10.00 I 1.55 .78 2.45	MAXIMUM EX AS DETERNI ITY REFINER or II Sect 10.00 10.00 10.00 10.00 10.00 10.00 9.50 9.50 9.50	TINCTION NED BY VI ENTS For I E12 20.45 20.25 20.20 20.20 20.20 20.60 20.60 20.60 20.60 20.60 20.60 20.60 20.60 20.60 20.75 11 1.95 .98 2.11	HALUES OF ISUAL METHOD 11 En 11 En 5 20.45 5 20.45 5 20.45 5 20.65 29.55 20.60 20.60 20.60 21.50 5
TABLE Plate 167 168 169 170 172A 172B 172A 172B 174A 174B Range Deviat % Nean V	B A COLF #2898 AND WE % Conc. (Correcte .C2285 .C2255 .C2255 .C2255 .C2255 .C2255 .C2255 .C2255 .C2255 .C2255 .C2250 .C2250 .C2250	ARISON OF AT 520 NU IGHT-DENS I Sect d) 10.00 10.50 10.50 10.50 10.50 10.50 10.50 10.50 10.50 10.50 10.00 9.25 10.00 9.25 10.00 1.55 .78 2.45 20.52	MAXIMUM EX AS DEFTER T ITY REFINER or II Sect 10.00 10.00 9.50 10.00 10.00 10.00 9.50 9.50 9.50	TINCTION NED BY VI ENTS FOR I E12 20.20 20.20 20.20 20.20 20.20 20.20 20.20 20.60 20.60 21.80 20.75 II 1.95 .98 2.11 20.62	HALUES OF ISUAL METHOD $\frac{11}{2}$ E $\frac{11}{2}$ E

TABLE A A COMPARISON OF MAXIMUM EXTINCTION VALUES OF #2898 AT 228 NU AS DETERMINED WITHOUT REFINE-MENTS BY TWO INDIVIDUALS

The sector settings at the isodensity points were then determined by use of the microphotometer. The $\mathbf{E}_{lom}^{1\%}$ values in table C show the corrected sector settings which were used with the uncorrected percent concentrations from table A.

Table D gives detailed information of the combination of the weight-density corrections used in conjunction with the sector settings as determined by the microphotometer.

The accuracy of the determination of the $E_{lom}^{1/2}$ of a substance depends upon the accuracy of $I_0/1$, c, and d. The cells used in the absorption work reported by this paper were accurately ground, hence this error is negligible.

Two factors determine the accuracy of the percent concentration of the solution. They are: (1) the accuracy with which the solute is weighed, and (2) the accuracy exercised in determining the volume of the solvent. The somi-micro calance which was used to determine the weight of the solute is accurate to plus or minus five thousandths of a milligram. Expressed in percent deviation this would be plus or minus five hundredths of one percent. This deviation is also negligible. The volume of the solvent was measured with a pipette. Since the density of the solvent and the volume of the pipette vary with the temperature, considerable deviation is to be expected. Hexane is unsuitable for pipetting because of its high vacor pressure.

TABLE C MAXIMUM EXTINCTION VALUES OF $\frac{1}{4}$ 2898 AT 228 MU AS DITERVINED BY THE MICROFUL CONSTER WITHOUT WEIGHT-DENSITY CORRECTIONS

Flate	Sector	Log Sector	% Conc.	e}%
167 168 169 170 172A 172B 172A 172B 172A 174A 174B	9.25 9.45 9.05 8.72 9.65 9.65 9.65 9.40 9.50	1.020 1.020 1.042 1.057 1.015 1.014 1.016 1.015 1.027 1.022	.02260 .02225 .02275 .02210 .02220 .02220 .02220 .02270 .02225 .02225	21.75 21.60 21.62 21.90 21.50 21.50 21.10 21.05 21.60 21.70
Range Deviati % " Nean Va	.8 on .4 1.2 lue 51.5	5 3 5 0		

Flate	Temp. ^O C	. Wgt. Hei gram:	kane Dei S	nsity V	olume Hexane ml.
167 168 169 170 172A 172B 172A 172B 174A 174B	22.0 22.4 22.5 22.2 24.2 24.2 24.2 24.5 24.5	12.1210 12.2127 12.0979 13.1114 13.0746 12.0746 12.0678 12.0678 12.0625 12.0625	. 50 . 50 . 50 . 50 . 50 . 50 . 50 . 50	616 1 620 1 619 1 618 1 608 1 608 1 607 1 605 1 605 1	9.82 9.95 9.78 9.66 9.78 9.78 9.78 9.78 9.78 9.78 9.77 9.77
Flate	Sector	Log Sector	₩ළt. Oil mළ	% Conc.	El [%] Elcm
167 168 169 170 172A 172B 172A 172B 172A 172B 174A	9.25 9.45 9.05 8.72 9.65 9.68 9.65 9.65 9.65 9.40 9.50	1.024 1.020 1.042 1.057 1.015 1.014 1.016 1.015 1.027 1.022	6.52 6.45 6.55 6.22 6.44 6.44 6.46 6.46 6.45 6.45	.02285 .02210 .02210 .02285 .02255 .02255 .02270 .02270 .02250 .02250	51.14 51.22 51.45 51.25 51.18 51.15 51.17 51.06 51.60 51.41
Range DeViat: % " Mean Va %	Elcm icn alue 21 Mean - H " - V Differen % Differen	Alues 52 27 56 26 Sector Se Photometer Visual 16e 16e	Mean - P: " Wa Differend %Differend ettings 9.40 9.95	Volumes ipette gt. Den. ce log Sec l.027 l.022 .025 2.5	20 Cm ² 19.79 .21 1.05 etor

TABLE D INTINCTION VALUES OF $\frac{1}{4}$ 2898 AT 220 MU AS DETERMINED BY THE EVERYDDE OF THE MICROPHOLOGENER AND ADIGHT-DENSITY

By k-ording the density of hexane at the room temperature* and also the weight of hexane used, it is possible to determine the accurate volume of herene.

This graph and the corresponding dutu** from which it was determined are accounted for these corrections. If the temperature-density line of the graph were extended to zero degrees centigrade, the density of the herme would be equal to .0752. The corresponding density from the international Oritical tables is .0705. This represents a difference of .15 percent from the accepted value. This difference may recalt from the slight impurities in the high quality connercial means used, and from the fact that the comparison value was interpolated, while the value quoted in the critical tables was actually determined at zero degrees centigrade. Table D shows a one percent difference in , volumes between the pipetted volume and the volume determined by the weight-density method.

By using the microphotometer to measure the ratio of the relative densities of the I_0 and I, the visual method of reading the plate is eliminated, and it is possible to interpolate between the sector readings of the two successive absorption bands. Table C shows that

** See Fages 15, 14

^{*} See page 12



Corrections Applied Buoyancy N = S + (Vm - Vs) -M = mass of corrected weight S = mass of weights Vm - volume of pyknometer Vs = volume of weights σ = density of air Density of Air = .00129 g/cm² S. T. P. = .000882 g/cm³ 740 nm; 24°C. At 17.69° Centigrade Volume of Pyknometer = 155.007 g of water 155064 g - 155.245 cm² IIW = S + (Vm - Vs)- 155.0674 + (155.2455 - 18.45) .000882 - 155.1678 g 155.1878 g = 155.5779 cm⁵ at 17.69⁰C. Volume of Fyknometer = 154.7920 cm⁵ at 4 ⁰C. = 102.6599 g Mh = 102.8599 + (155.5779 - 12.2) .000882Hexane M = 102.9852 g Dh **=** 102.9852 154.79.0 = .6655 at 17.69⁰ Centigrade

DENSITY OF HEXAME (CONT)

At 25.00° Centigrade Volume of Fyknometer = 154.8290 g of water 154.8290 g = 155.2786 cm² Nw = 154.8290 + (155.2786 - 18.2) .000882 = 154.9498 g 154.9498 g = 155.5284 cm² at 25⁰ Centigrade Volume of Fyknometer = 154.3652 cm³ at 4⁰ Centigrade Hexane = 101.7771 g - 101.7771 - (155.5284-12.1) .CCC862 Mh = 101.9025 g $Dh = \frac{101.9025}{154.0622}$ = .6601 at 25.00° Centigrade At 33.52° Centigrade Volume of Fyknometer = 154.4406 g of water 154.4456 g - 155.2889 cm³ MW = 154.4456 + (155.2889 - 18.1) .000882= 154.5655 g 154.5655 g = 155.4154 cm² at 25.52⁰ Centigrade Volume of Fyknometer = 152.7198 cm² at .2.52⁰ Centigrade Hexane = 100.54c5 g - 100.5465 + (150.4154-11.9) .000682 Mh = 100.6724 gDh = 100.6724 152.7198 - .6549 at 22.52° Centigrade

it is possible to reduce the percent deviation of the maximum extinction values of $_{\mu}$ CSSE by using the microphotometer to determine the maximum extinction points.

Due to limitations of the scale on the adjustable sector, it is impossible to set any two successive exposures closer than one half of a sector setting. This means that the maximum extinction value of the oil could deviate plus or minus one percent before the deviation of one half of a sector setting is perceptible.

After a series of 40 determinations, Ewing, Vandenbelt, Emmett, and Bird (1) report a near $E_{lom}^{1/0}$ value for fittee of 21.46. Their percent of deviation is three and one half. By using methods similar to those used in the Ewing <u>et al</u>, determinations, the values of table A were obtained. This table gives the $E_{lom}^{1/0}$ values of 20.78, and 20.92 respectively. The differences between the Ewing <u>et al</u>, values and those given in table A may seem large. However, it has been shown by Escheile (12) and Embree (2) that the $E_{lom}^{1/0}$ values of a vitamin A oil decrease with the passage of time, and the type of solvent used will affect the results. The solvent used in this study is hexane; Ewing <u>et al</u>, used isoproxyl alcohol.

It can be seen by comparing table A with table D that the percent deviation in the measurement of $\frac{1}{4}$ LE98 can be reduced considerably by using the refinements of table D. It is found by comparing the sector settings

determined visually (table A) with those determined on the microphotometer (table D) that the latter are consistantly lower. Taking mean values, the log values of the sector settings, as determined on the microphotometer, are, when compared, about two and one half percent lower than the log values of the sector settings determined visually. A possible explanation of this fact is that the eye not only sees the extinction point at 226 mu, but is influenced by the relative densities of the band on either side of 228 mu. In other words, the eye is not as selective as the microphotometer.

Table B indicates that by using the weight-density method without the refinement of the microphotometer, the $\mathbb{E}_{1\text{ cm}}^{1/2}$ values tend to be lower than those obtained without using special precautions. The errors introduced by pipetting tend to make the extinction values slightly higher, and the visual spotting tends to make these extinction values somewhat lower. Therefore there is a slight compensation of errors. It must be concluded that if the refinements are to be effective, both must be used.





Summary;

1. It is possible to interpolate between the sector readings of two successive absorption bands by using the microphotometer to measure the relative densities of I_0 and I. When such interpolation is used, the percent deviation is reduced considerably.

2. The sector settings as determined by the microphotometer are consistantly lower than those determined visually.

2. By using the weight density method of determining volume, the one percent error resulting from the determination of volume by pipetting is eliminated.

4. If the refinements mentioned in the previous conclusions are to be most effective, they should be used simultaneously.

5. By applying these methods, the maximum extinction values tend to be higher than those obtained when no corrections are applied.

References:

- 1. Baxter, J. G., and Robinson, C. D., Science, <u>92</u>, 200 (1940)
- 2. Embree, N. D., Ind. Eng. Chem., Anal. Ed., <u>12</u>, 144 (1941)
- Ewing, D. T., Vandenbelt, J. N., Emmett, A. D., and Bird, C. D., Ibid <u>12</u>, 629 (1940)
- 4. Hogness, T. T., Zschelie, F. F., and Sidwell, A. E., J. Fhys. Chem., <u>41</u>, 179 (1927)
- 5. Holmes, H. F., and Corbet, R. E., J. Am. Chem. Soc., <u>89</u>, 2042 (1957)
- 6. Mc Farlan, R. L., Bates, P. K., and Merrill, C. E., Ind. Eng. Chem., Anal. Ed., <u>12</u>, 645 (1940)
- 7. Mead, T. H., Biochem. J., 52, 589 (1939)
- 8. Mitchell, J. H., Jr., and Kraybill, H. R., Ind. Eng. Chem., Anal. Ed., <u>15</u>, 765 (1941)
- 9. Lunch, R. H., J. Am. Chem. Soc., 57, 1865 (1955)
- 10. Wilkie, J. B., Ind. Eng. Chem., Anal. Ed., 10, 209 (1941)
- 11. Zscheile, F. P., and Comar, C. L., Bot. Gaz., <u>102</u>, 462 (1941)
- 12. Zscheile, F. P., and Henry, R. L., Ind. Eng. Chem., Anal. Ed., <u>14</u>, 422 (1942)





