AN INVESTIGATION OF THE ABSORPTION SPECTRA OF SOME FISH OILS AND OTHER EXPERIMENTS

by

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C O N T E N T S

An Investigation of the Absorption Spectra of Some Fish Oils

The Ion Content of The Air

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FIGURES AND TABLES

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AN INVESTIGATION OF THE ABSORPTION SPECTRA OF SOME FISH OILS

INTRODUCTION.

Since it has been definitely established that the determination of the intensity of absorption of Vitamin A concentrates and liver oils of high Vitamin A potency in the ultra violet at 3280 A^{o} gives values which agree with the biological test[1], it is possible that the method might somehow be extended to oils of lower potency without any loss of accuracy.

This report deals chiefly with such an attempt, the oil under investigation being British Columbia Pilchard body oil. Several methods are outlined for obtaining the extinction coefficient at 3280 A^{o}; the absorption spectrum of the oil is obtained and investigated. The discrepancy between biological tests and physical tests is discussed.

Included in the report are the details of a method for obtaining the ultra violet absorption spectrum of any solution with only one photographic exposure.

1. Coward, Dyer & Morton. (1932) Biochem. J, 26, 1593.

EXPERIMENTAL

The extinction coefficient u is defined by the equation $I = I_0 10^{-ux}$, where I_0 is the light incident on the absorbing solution and I that which is transmitted through x cm. of the solution. The value of this coefficient at 3280 A^0 was determined by two distinct methods. The first is a visual method using a fluorescent screen; the other the photographic procedure of comparison of intensities. In the methods given below, the last three are photographic.

Method I.

The most convenient way of determining u includes the use of a copper arc, a filter, a fluorescent screen and some mechanism for controlling the intensity of the incident light. Such a method has been patented by Adam Hilger Ltd. and the apparatus named a "Vitameter A".

Following this principle an instrument was designed and used for the Vitamin A tests. It is shown in diagram in Fig.1. A is a copper arc run at 3 amperes, on a direct current circuit of 110 volts; B_1 and B_2 are two small circular apertures; C is a light filter which transmits only the radiation near 3280 A^0. D is a cell containing the solution unter examination; E is an adjustable sector and F a fluorescent screen of Canary glass.

A balancing glass plate G is placed in the beam which does not pass through the solution.

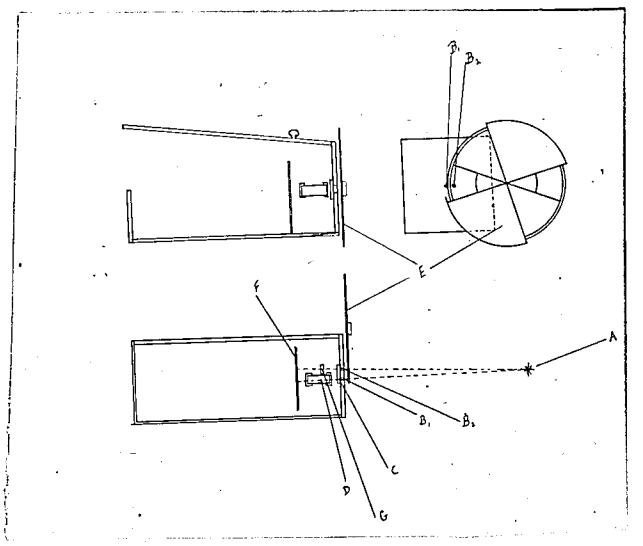


Fig.1.

By varying the adjustable sector the intensity of the light passing through B_2 can be varied and the two images of F can thus be brought to the same brightness. From the angular setting of the sector $\log \frac{I_0}{I}$ can be found and hence u.

The balancing plate G was necessary to compensate for the loss of radiation by reflection and absorption caused by the windows of the cell. While quartz windows would transmit more light at 3280 A°, it was found that glass windows made from photographic plate would decrease the intensity very little. Moreover, G corrects for any error that might arise because of the

absorption.

The filter C was a quartz window, sufficiently silvered so that one could just see the outline of a window when looking through the filter.

If the film is too thick it cuts down the intensity in the region of investigation. If it is too thin it is not sufficiently selective. Fig.2 shows the effect of the filter on the radiation from a copper arc.

The sector was run at a speed of 30 revolutions per second, sufficiently high so that there would be no flicker and Talbot's law[2] would then hold.

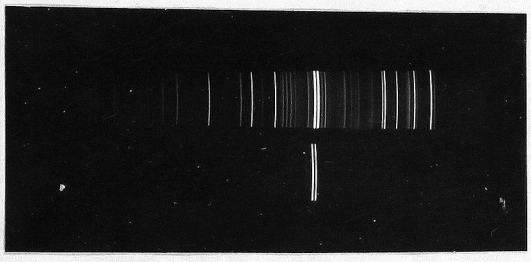


Fig. 2.

2. "The apparent intensity of an intermittent light is proportional to the time of each individual flash, provided there is no flicker."

Method II.

The photographic method for comparison of intensities requires the "calibration" of each photographic plate because the relation between the intensity of the incident light and the blackening of the plate is so complex that it can not be determined by formula. It is necessary, therefore, to obtain experimentally a curve showing this relation. (See Fig. 3) Knowing the density or blackening produced by an unknown intensity, the latter can be directly read from the graph.

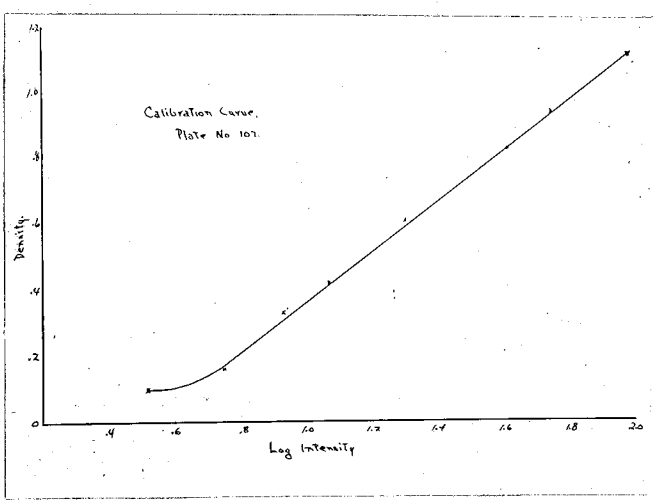


Fig. 3.

Since the relation mentioned above is a function of λ, the wavelength, it is necessary to have calibration curves for every wave length for which u is to be determined. This method, however, concerns only monochromatic light of λ = 3280 A^{o}. A small Hilger quartz spectrograph was used as a monochromatic illuminator by masking the dark-slide opening so that only the radiation of the two strong lines in the copper arc at 3247 and 3274 A^{o} would be exposed to the photographic plate.

For calibration purposes, a steady light is very necessary, unless the calibration is done in one exposure (see Method IV.) For this reason, an argon glow lamp of the type which is used in the study of minerals by fluorescence, was employed. An image of the source was focussed on the slit of the spectrograph by means of a quartz condensing lens. Intensities were varied in known ratios by means of meshes placed adjacent to the condensing lens. When placed at an appreciable distance from the lens, it was found that they caused shadow effects in the illumination of the slit. There was no trace of such a disturbing effect when the screens were placed close to the lens.

The transmitting power of the screens was measured by placing a photo-electric cell in the position occupied by the slit of the spectrograph. The cell was

first tested to see if it gave a linear relation between the photo-electric current and intensity by using the inverse square law. The transmitting powers of the screens were: #1, 56.8%; #2, 41.2%; #3,20.4%; #4,5.71%. Using these singly and in combinations it was possible to cut the original intensity, arbitrarily designated as 100 down to 1 in eight or nine steps.

Fig. 4 shows a typical plate. The radiation used for calibration was that at the head of a band in the spectrum of the glow lamp at approximately 3350 A^{o}. The lower strip showing exposures of the main lines in the copper arc were obtained by placing the absorption cell immediately in front of the upper half of the slit, so that the radiation falling on this part of the slit would have passed through the absorbing solution. Intensities of the absorbed and unabsorbed radiation and hance u, were then determined from the calibration curve. All exposures were for a constant time, 30 secs.

Eastman 33 plates were used and were developped in Rodinal which had less tendency to fog the plate than the ordinary pyro developers [3]. While in the developer the plate was kept moving vigorously. In using a camel-hair brush to obtain even development it

3. G.R.Harrison, J.Opt. Soc. of Amer. Mar.1934.

was found that the emulsion was usually scratched in the process.

Density measurements were made with a Moll recording microphotometer.

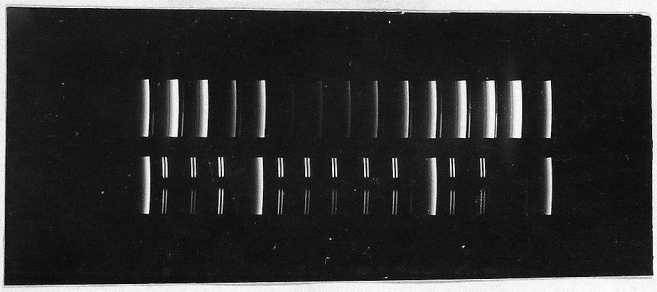


Fig. 4.

Method III.

A sector photometer (Adam Hilger Ltd.)[4] was used to determine the absorption spectrum and in particular the absorption coefficient at 3280 A°. A tungsten-steel spark was used as a light source.

4. Catalogue of the Manufactures of Adam Hilger Ltd.

Fig. 5 shows a spectrogram taken by this method.

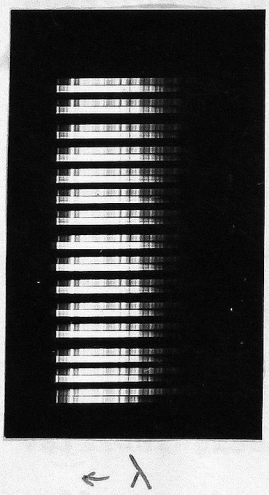


Fig.5.

Method IV.

This remaining method involves the use of

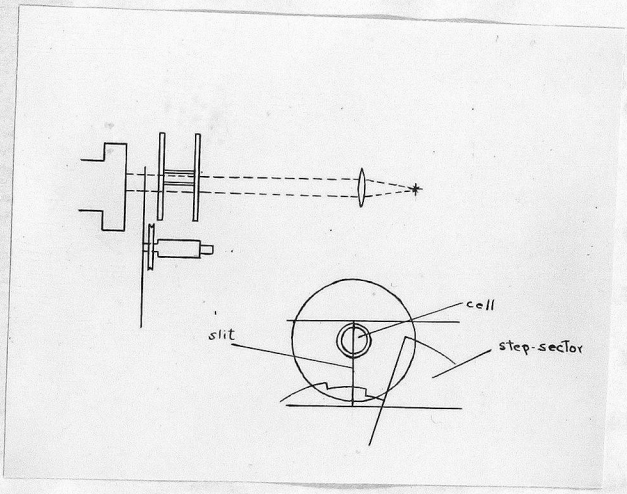


Fig.6

a step-sector for calibration purposes. Fig.6 illustrates how the absorption spectrum of a solution and calibration intensities can be obtained simultaneously on a photographic plate. Light from a point source was condensed upon the slit of a quartz spectrograph (Hilger E1) by means of a sphero-cylindrical lens such that light remained parallel in the vertical plane. Therefore, when the cell was placed adjacent to the slit in the path of the light, the horizontal portion of the wall of the cell cast a distinct shadow on the slit. The spectrum was therefore divided into two portions, the upper part being the light which passed through the cell and the lower part being the unabsorbed spectrum, as in Fig.7.

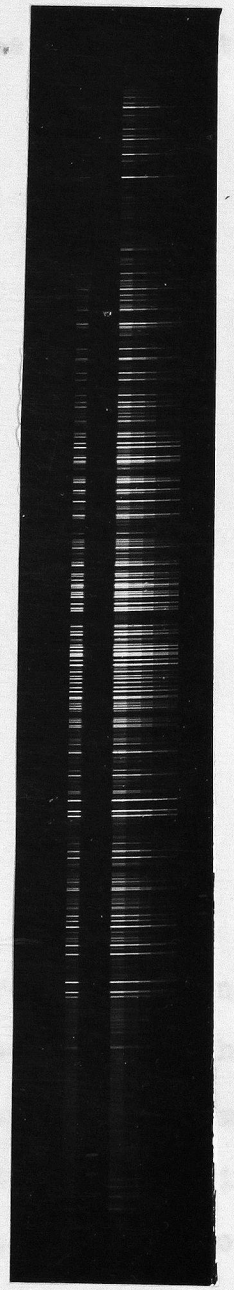


Fig.7.

It is necessary in this method to have even illumination all along the slit, except where the shadow of the cell wall is, before the absorbing material is introduced into the cell. Cyclo-hexane was the solvent

used and since it has negligible absorption in the region under investigation, and since quartz windows were used for the cell, (Fig.8) any difference of illumination between the two portions of the spectra would be due to

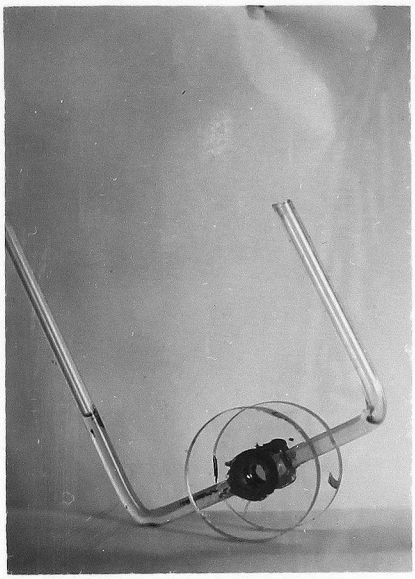


Fig. 8.

reflection on the cell windows. Whereas in the upper beam, when the cell is loaded, there is only one reflecting surface of importance, while in the lower path there is two, there will undoubtedly be an error arising which can be overcome by methods suggested below.

To ascertain whether or not this error was of importance a photograph was taken with the cell

filled with solvent only and the densities of the two spectra compared on the microphotometer. Fig.9 shows the negligible result.

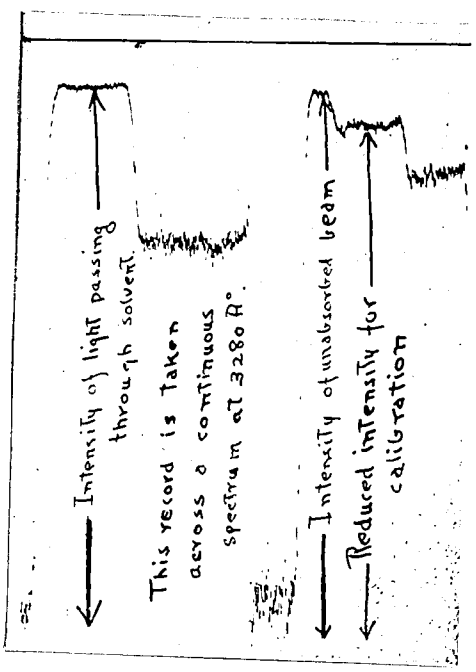


Fig.9.

However, to overcome even this small error it would be necessary to design a double cell which had two compartments, one for the solution, one for the solvent. This would then compensate for the reflection.

The introduction of a step-sector[5] between the slit and the cell, and the adjustment of the cell so

5. G.R.Harrison, J. Opt. Soc. of Amer. Mar.1934.

that about 2/3 of the slit length can be used for calibration intensities, gives a final photograph similar to Fig. 7.

Using the iron arc as a source, it is possible to obtain u for a sufficient number of wave-lengths to plot the absorption spectrum. A small, sharp absorption band of width less that 25 A^{o} might not be detected in some regions. Using a continuous source, this difficulty would disappear.

The windows of the cell in Fig.8 are much larger than necessary. Rather than cutting them into a more convenient size, they were cemented on as they were. Different cements were tried for this purpose. As the cell had to be dismantled frequently to clean it, it was found best to use "liquid solder" which does not harm the polished surface of the window. This cement is also insoluble in chloroform, ether and cyclo-hexane.

In preparing the solutions, several precautions were found necessary. Pippettes were re-calibrated because of the viscous oil. Solutions should be allowed to stand overnight. When this precaution was not taken, that is, when readings of the extinction coefficient of a solution were taken soon after its preparation, it was found that different portions of the solution had different densities. The top portion in the test-tube had a smaller coefficient than the next,

Fig. 9a. Diam. of sector - 3.5"

while the solution at the bottom of the tube was very much more dense. This resulted even though the solution had been shaken immediately after its preparation.

To be certain there was no contamination from the corks, solutions were always prepared in 20 c.c. glass-stoppered bottles.

Microphotometer readings were done in the usual way using $D = \log \frac{I}{I_0}$ for the definition of intensity.

The step-sector used in method IV. for calibration is shown in Fig. 9a. It was constructed so that both the light beams would be intermittent. The use of sectors for the reduction of intensity for photographic work has been proved to cause no errors.[6]

The oxidation of the oil samples was performed by bubbling air throughtthe oil for 30 hours. In oxidizing the sample to obtain curve d, Fig.13, air was bubbled through the oil at 60° C. for six hours and exposed to the radiation from a copper arc for eight hours.

To extract the pigment of the pilchard oil, the oil was first dissolved in cyclohexane, a little diatomaceous earth added and the mixture shaken. Absorption measurements were taken after filtering.

The extraction of the unsaponifiable fraction followed the procedure given by Baumann and Steenback.[7]

6. G. R. Harrison, J. Opt. Soc. of Amer. Mar. 1934.
7. C. A. Baumann & H. Steenbock, J. Biol. Chem., 101, 547-60,1933.

RESULTS

Method I.

Several samples of pilchard oil were studied, one or two reference cod-liver oils, and a standard solution of Vitamin A obtained from British Drug Houses Ltd. To obtain the Vitamin A content in terms of 1934 International Vitamin A units, the extinction coefficient for a one-percent solution of 1 cm. length was multiplied by the conversion factor 1600.

To prove the consistency of the measurements, readings for different concentrations of the same oil were taken and a graph plotted to illustrate Beer's law, which postulates a linear relation between the extinction coefficient and concentration. Fig. 10 is such a graph.

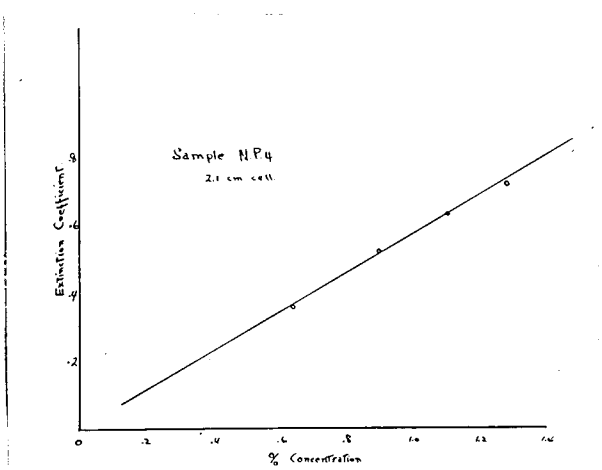


Fig.10.

Table I.

Oil	% Concentration	Sector Reading	Absorption Coefficient	Vitamin A Content
Standard	.045	18.5	.69	11,700
CL 1	1.0	14°	.81	620
NPC-1	1.0	15½°	.76	580
NP 5	.43	38° V		
NP 5	.83	21° V	.80*	610
"	1.11	11.5° V		
"	.48	39°		
"	.71	26°		
"	.96	16°	.77*	590
"	1.20	11°		
"	.83	20°		
"	1.12	12°	.81*	620
"	1.37	6°		
"	1.05	14°	.77	590
NP4	.83	27° V		
"	1.10	19° V	.58*	440
"	1.40	13° V		
"	1.05	23°	.59	450
NP 7	1.05	26½° V	.53	385
"	.75	35°		
"	1.05	27°	.50*	380
"	1.29	18°		
"	.83	32°	.52*	400
"	1.40	16°		
NP 12	1.0	21°	.61	460
"	1.0	22°	.59	450
"	1.0	22°	.59	450
WCI-1	1.39	19°		
"	1.04	28°	.48*	370
"	1.05	26°	.52	400
"	1.05	27°	.50	380
WCI-1 (oxidized)	1.0	14½°	.79	600
WCI-1 (in room)	1.0	22°	.61	460
NPC-1(oxid.)	1.0	15°	.77	590

Absorption coefficient values in Table I. are for a cell of length 2.1 cm. Those marked * are average values for 1% solutions obtained from graphs similar to Fig. 10.

Method II.

This method was not used for Vitamin A measurements because of its inconvenience compared with Method I. However, absorption measurements were taken which showed the accuracy of the procedure.

As in Method I., a graph was obtained for two different samples of cod-liver oil, showing the linear relation between concentration and absorption. See Fig. 11.

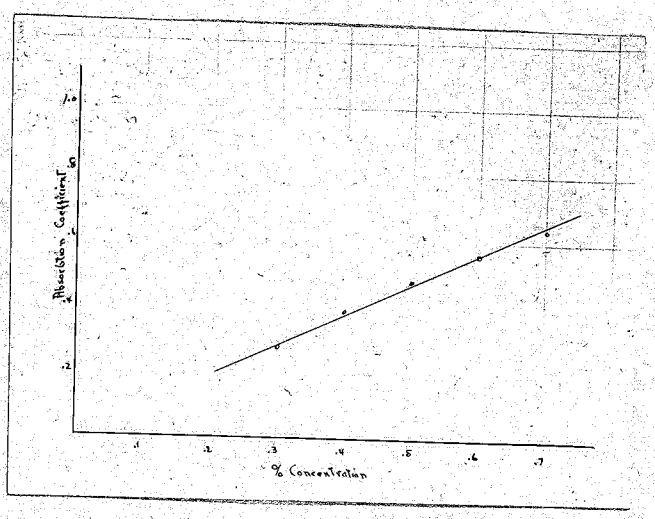


Fig.11.

Method III.

This procedure was adopted mainly for the purpose of checking the results of Method I. The

following table will show how the two different experiments give the same results.

Table II.

Oil	Plate No.	% Concentration	Extinction Coefficient Meth. III.	Meth. I.
NP 7	1	.75	.32	.34
"	1	1.0	.49	.51
"	1	.43	.22	.21
"	2	.43	.23	.21

METHOD IV.

In the manner described it was found possible to record conveniently on a photographic plate the absorption spectra of several samples of oil. For reasons pointed out below, the following absorption spectra were obtained:

a. Pilchard Oil. N.P.C.1.

b. " " " - pigment extracted with diatomaceous earth.

c. Unsaponifiable fraction of Pilchard oil, W.C.I.1

d. " " of oxidized Pilchard Oil W.C.I.1

These are shown in Figs. 12 & 13.

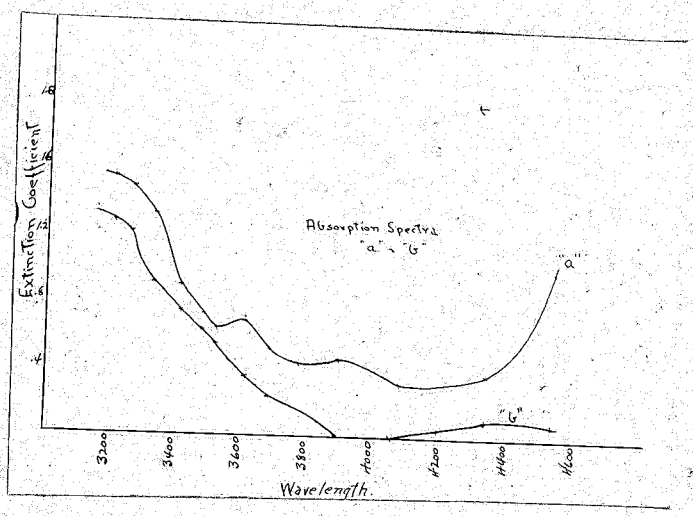

Extinction Coefficient
Absorption Spectra
"a" & "b"
"a"
"b"
3200
3400
3600
3800
4000
4200
4400
4600
Wavelength.

Fig. 12.

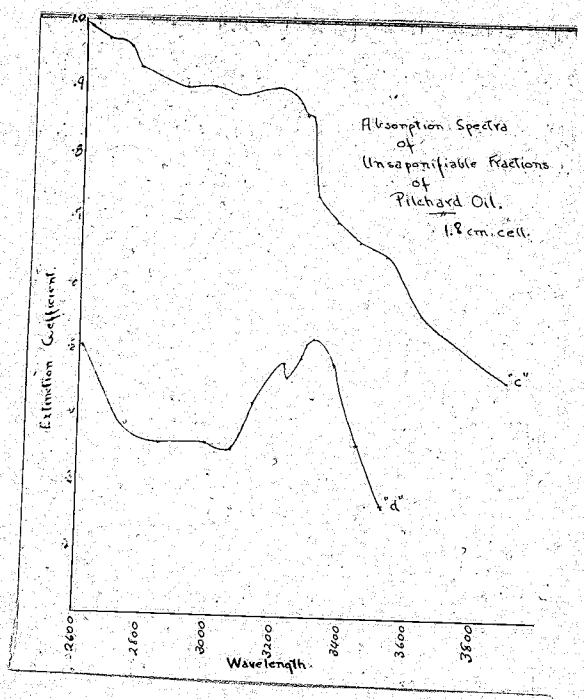

Absorption Spectra
of
Unsaponifiable Fractions
of
Pilchard Oil.
1.8 cm. cell.
Extinction Coefficient
"c"
"d"
2600
2700
3000
3200
3400
3600
3800
Wavelength.

Fig. 13.

DISCUSSION OF RESULTS.

Because the first procedure described in the earlier part of the report involves no photography and gives readings direct, it is by far the most convenient method. It not only gives consistent results (see Fig.10), but also checks with the photographic method according to Table II.

However, if the values of the Vitamin A content obtained by Method I are compared with biological assays made by the Biological Board of Canada, it is found there is a large discrepancy between the results of the physical and biological methods, as shown in Table III.

TABLE III.

	Spectroscopic	Biological
W.C.I.-1	380	1200
N.P.C.-1	580	175

Furthermore, the usual value of pilchard oil, from rat tests, ranges from 100 - 200 Int. units of Vitamin A per gram. Therefore Method I, and indeed any spectroscopic measurement, since Method I has been shown to give readings consistent with other methods, gives incorrect values of the Vitamin A content.

This is to be expected since in oils of low potency the contribution to the total absorption by other

materials in the region of 3280 A° is of considerable importance. Since it is hardly possible that this irrelevant absorption is related to the Vitamin A content or that is constant in all pilchard oils, no correction factor can be used to allow for it.

In an effort to explain this extra absorption it was considered that it was due to the free fatty acids in the oil, the pigment, or some other unsaponifiable substance. The examination of the two spectra in Fig. 12 shows that while extraction of the pigment cuts down the absorption in the visible by 80%, there is not an equal diminution of the coefficient at 3280 A°. This proves that the pigment is not responsible for the bulk of the absorption in this latter region. In decolorizing the oil, the vitamin may have been partially extracted, which would cause a decrease in the absorption in the ultra-violet; the diatomaceous earth might have absorbed a little of the oil, which is more possible since the decrease is not confined to the Vitamin A region; or the pigment may have some slight absorption in the ultra-violet.

Fig.13 shows the results of an investigation to determine the importance of absorption by the unsaponifiable fraction. From curve "c" it is seen that the Vitamin A band is on the edge of a large absorption band

which extends down into the ultra-violet. It is this band that causes the increased absorption at 3280A°. This has been found in other oils of low potency.[8]

Measurement of the Vitamin A content of pilchard oil by a direct reading of its extinction coefficient is therefore impossible. However, if the Vitamin A could be eliminated completely from the oil and the extinction coefficients, before and after removal, compared, a quantitative determination should be able to be made.

With this in mind, the oil was oxidized, as described, and the unsaponifiable fraction studied. Curve "d", Fig.13, shows the result. While there is a difference in the absorption coefficient at 3280A° between curves "c" and "d" equivalent to 180 Vitamin A units, there has also been a decided change in the shape of the whole curve. This must be due to the destruction of the unsaponifiable materials other than Vitamin A. Because of this it is difficult to make any definite conclusion concerning the Vitamin A content. Before the spectroscopic method of Vitamin A determination in Pilchard Oil can be used, it will be necessary to devise means for removing the Vitamin A completely without destroying any of the other unsaponifiable materials in the oil.

8. R.A. Morton & I.M. Heilbron, Biochem. J., 1928, 22, 987

It should be noted here that absorption measurements of the oxidized oil itself are higher than that of the fresh oil. (see Table I.) An oil which had been kept at room temperature for one month instead of in a refrigerator showed a 20% increase in absorption although the container ~~in which it~~ was tight corked. This increase is due to increase of the fatty acid content of the oil.

Measurements of a cod-liver oil and a Vitamin A standard showed the reading of the extinction ceefficient was a reliable method for Vitamin A assay. The Vitamin A standard was guaranteed to contain 12,500 Int. units per gram. The value according to Method I was 11,700. This is as correct as could as could be expected.[9] The cod-liver oil had been tested biologically and showed a value of 600 Int. units. These two results compared with the previous ones on pilchard body oil show the necessity for a new method for a physical determination of the Vitamin A content of low potency oils.

9. Lathbury, Biochem. J., 1934, 28, 2254

THE ION CONTENT OF THE AIR

INTRODUCTION

Many experimenters have found that the measurement of very small direct currents by amplification with thermionic tubes is more convenient and just as accurate as the old style electrometer method.

This report shows how the General Electric "FP-54 Pliotron" has been used for the measurement of extremely small direct currents in the study of the ion content of the atmosphere. The method is fundamentally the same as that of Koller[1] but with several modifications. The tube was also used to measure a capacity of the order 30×10^{-12} farads.

Included in this paper are the readings taken over a period of one month to show the variation of the ion content with atmospheric conditions.

EXPERIMENTAL

The usual method for measuring the ion content of the air is the "aspiration" method.[2] It consists of an aspirator through which the air is drawn at a known speed. An electric field in the tube forces the negative or positive ions on a collector and the

1. Koller - Jour. Franklin Inst. Nov.1932.
2. "The Electrical Conductivity of the Atmosphere and its Causes" - Victor F. Hess. Constable, London.(1928) p.24.

quantity of electricity thus collected is measured by the change in voltage of the collector. In using the FP-54 the collector is connected to the control grid and allowed to "float" for 30 or 60 seconds. The change in voltage can be determined from the tube characteristic.

The tube specifications given by the manufacturers are as follows:

Filament	-	2.5 volts, 0.09 amperes.
Plate	-	6 volts.
Central grid	-	-4 volts.
Space charge grid	-	4 volts.
Input resistance	-	10^{16} ohms.
Plate current	-	40 microamps.

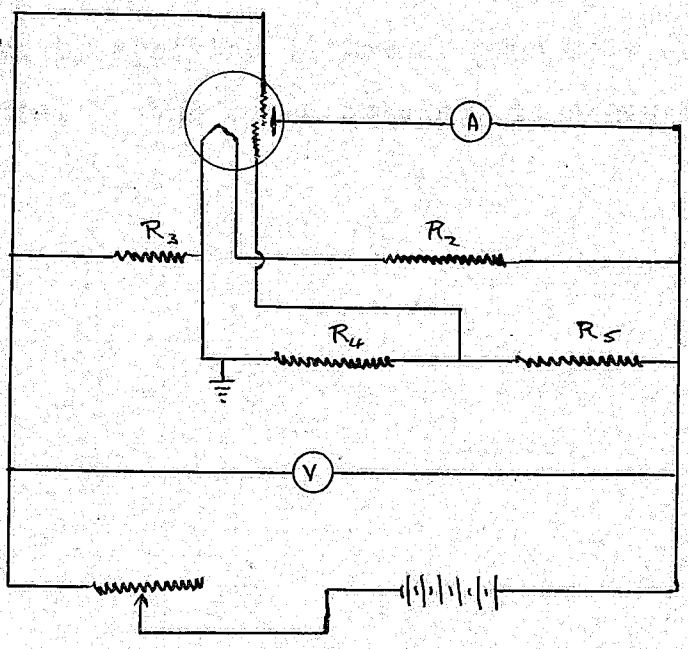


Fig.1

The circuit used is shown in Fig.1. It was found that the potential drop across the filament was 2.34 volts when the filament current I_f was .090 amps. R_2 must then be 40.7 ohms to make the plate voltage 6v with respect to the negative side of the filament. R_4 was made twice R_5 so that the potential of the space charge grid would be + 4 volts. So that the current flowing through R_4 and R_5 would be negligible R_4 was made equal to 1000 ohms and R_5 500 ohms. The total current going through R_3 will therefore be .094 amps. To get a grid voltage of -4 volts R_3 should be 42.5 ohms.

The grid voltage-plate current characteristic was obtained by applying different grid voltages with a potentiometer. The curve obtained is shown in Fig. 2.

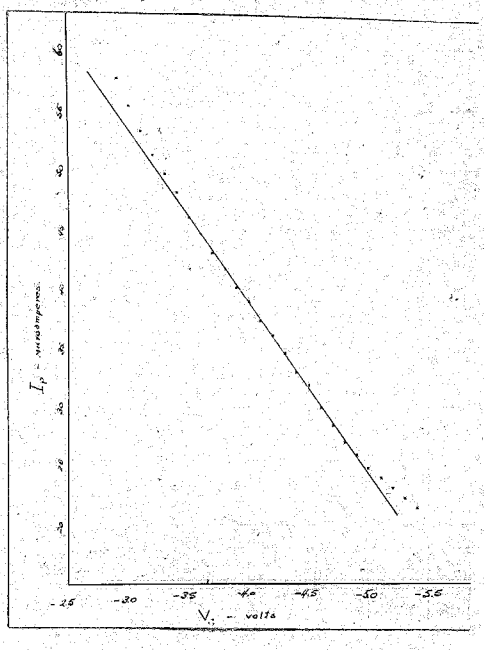


Fig.2

Fig 3 shows how the ion collector was connected to the grid of the tube. A clockwork automatically set the grid circuit at -4 volts every 30 seconds.

The plate current was measured with a mirror galvanometer of low sensitivity. The regular current of 40 microamperes was balanced out with a dry cell and variable resistance.

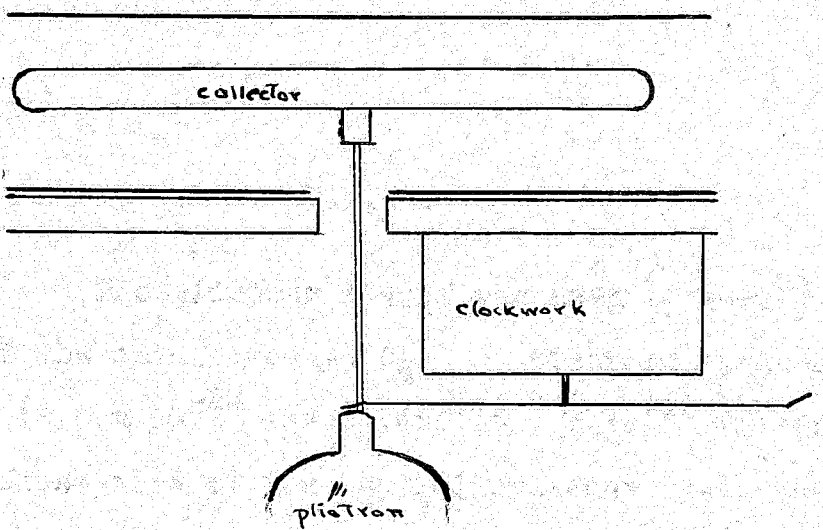


Fig. 3.

In later work the collector in Fig. 3 was redesigned so that there would be no potential barrier at the entrance of the aspirator. The method follows that of Swann [3]. Fig. 4 shows the arrangement.

3. Terr. Magn. 19, 171 (1914)

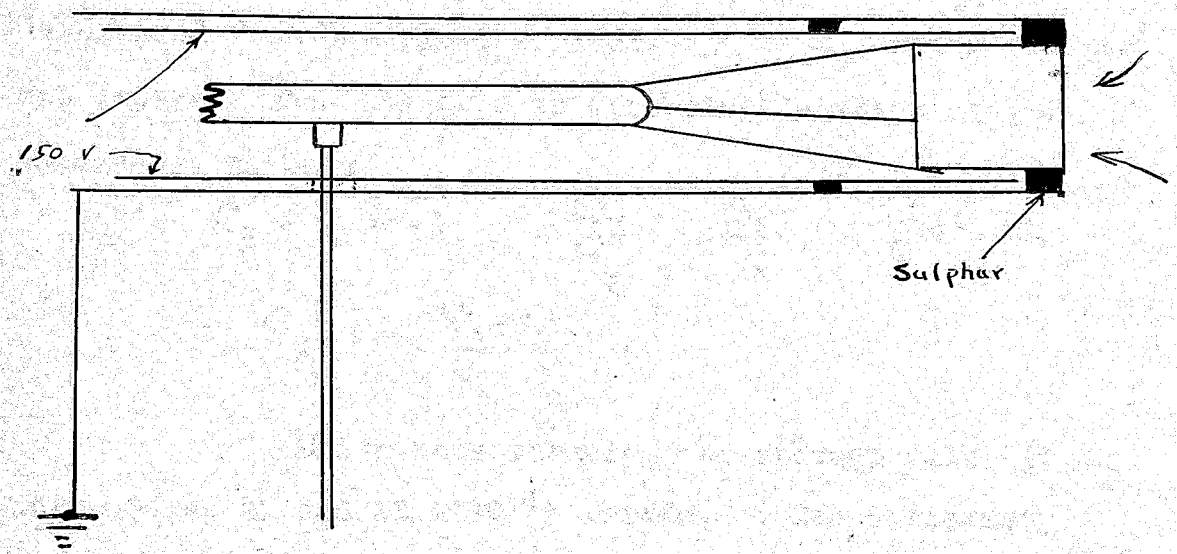


Fig. 4.

The pliotron itself was used to measure the capacity of the grid circuit, C_g. Standard condensers of capacities 42.5 x 10^{-12} farads and 15.2 x 10^{-12} farads were built. These were of the coaxial cylinder type, the outer cylinder was 10 cm. long and had a radius of 0.54 cm. Sulphur was used as a dielectric and all tests were made while the sulphur was still quite fresh. Capacities were calculated directly from the formula,

$$C = \frac{K1}{2 \log r_a/r_i}$$

The method of finding C_g was as follows: The standard condenser of capacity C_s was charged and then

Connected to the grid. The resultant voltage V_r was determined from the plate current. If C_r is the resultant capacity when the grid is connected to the standard condenser, we have

$$C_g + C_s = C_r, \quad \frac{Q_g}{V_g} + \frac{Q_s}{V_s} = \frac{Q_g + Q_s}{V_r}$$

$$\therefore C_g = \frac{V_s - V_r}{V_r - V_g} C_s .$$

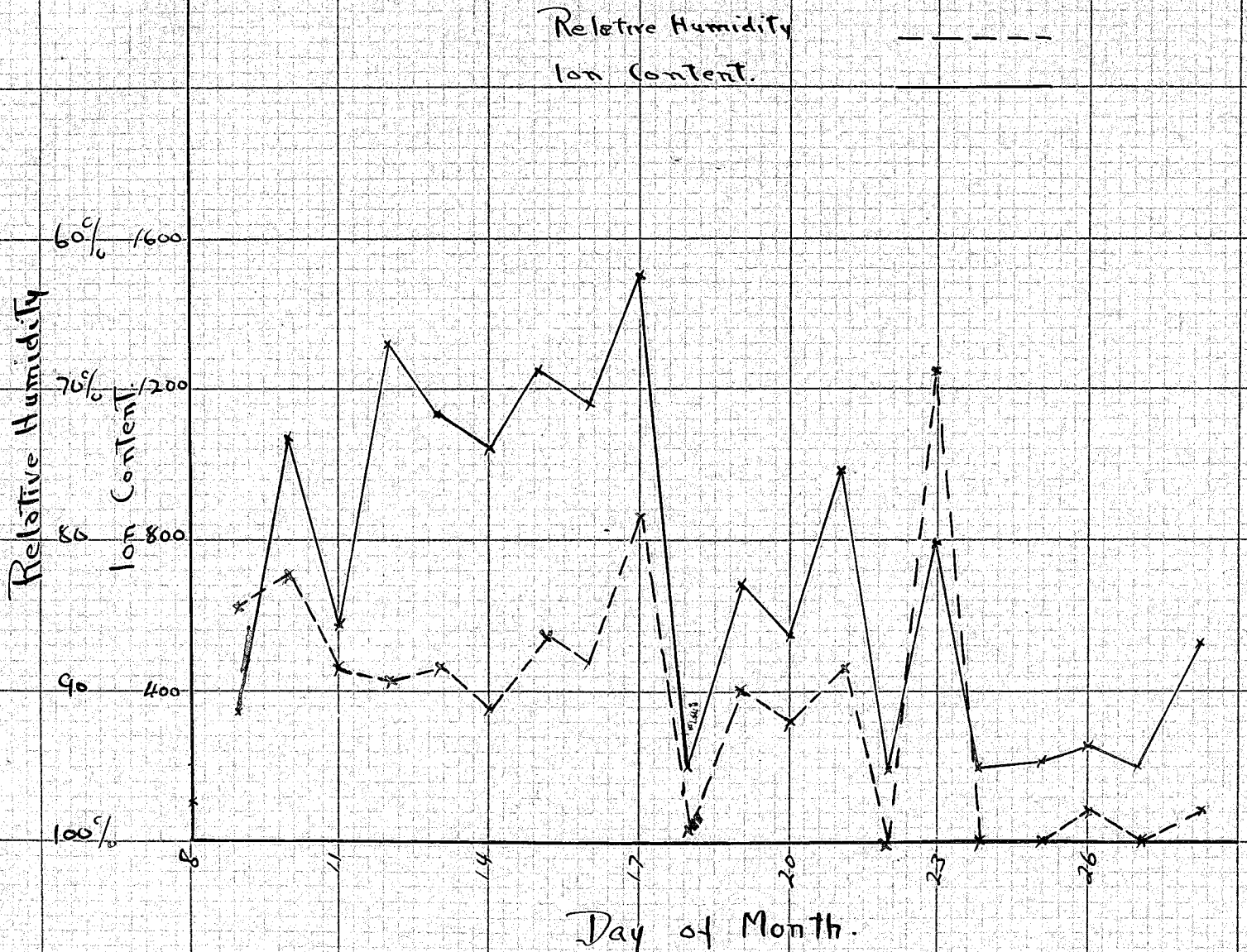
Using this formula, the average value of C_g was between 31 and 32 x 10^{-12} farads. Two different standard condensers were used to obtain a check on the method They were mounted vertically and could be connected to the grid by swinging them about their own axes until a small wire-arm joined to the inner cylinder touched it.

Air was drawn through the collector by means of a fan driven by an electric motor at a velocity of 3150 cc per sec. This value was determined with an anemometer. Taking the mutual conductance M from the characteristic curve as 1811 microamp/volt, then we can calculate the number of ions per cc. per microamp. change in I_p per 60 secs.

This number

$$= \frac{C \times 3 \times 10^9}{M \times 3150 \times 60 \times 4.77 \times 10^{-10}}.$$

$$= 58 \text{ ions per cc.}$$


Relative Humidity
Ion Content.
60%
70%
80
90
100%
1600
1200
800
400
Relative Humidity
Ion Content
8
11
14
17
20
23
26
Day of Month.

RESULTS

Daily readings of humidity and ion content show a close relation to each other. This is shown in Fig. 5.

Because of the presence of a large number of droplets in the air when the humidity is high, ions will tend to collect on these and become "large ions" of low mobility which are not collected by the apparatus.

Daily readings beside an open window were taken over a period of two months. No relation between any weather conditions, except humidity, and the ion content could be found. The average ion content over the period was 520 ions per cc. The values ranged between 180 cc and 1600 cc.

The presence of the experimenter in the room in which readings were taken would tend to decrease the ion content. Room readings were always considerably lower when the window was shut than when it was open except on exceedingly moist days.