1933

A magneto-optic method of determining the vitamin content of various substances

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UMI®
A MAGNETO-OPTIC METHOD OF DETERMINING THE
VITAMIN CONTENT OF VARIOUS SUBSTANCES

BY

Gerrit M. Wissink

A Thesis Submitted to the Graduate Faculty for the Degree

DOCTOR OF PHILOSOPHY

Major Subject: Physics

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1933
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I. INTRODUCTION

Ever since the discovery of vitamins much research and investigation have been done to isolate or separate the vitamin from the food containing it. It is known that certain foods contain certain vitamins, but there is still some question whether anyone knows exactly just what part of the food contains the vitamin itself. Much work has been done by scientists in trying to find the vitamin content of various foods.

The biologists, in trying to determine the value of this quantity, have fed animals on diets and noted the physiological differences. For example: one group of rats or guinea pigs would be fed on a diet containing vitamin A while a similar group would be fed on a diet deficient in vitamin A. The differences in the two groups would be noted at regular intervals while the test was in progress; and consequently, much time would be needed to carry on a test of this kind. Furthermore, much difficulty has been experienced in keeping the two distinct groups of test animals under identically the same conditions throughout the test; and consequently, several tests would have to be made before a definite conclusion could be reached.
During the last several years, attempts have been made to find a more rapid method than the biological method for finding the vitamin content of different foods. Chemists have worked to find the chemical constitution of vitamins and to isolate the vitamins from the foods in which they were found. The physicist has used different methods, among which the colorimetric and spectrographic are the outstanding,

This recent work has shown that there is some correlation between the biological and physical tests, but there is no definite proof that the latter are absolutely dependable in all cases,

The purpose of this investigation was to find another physical method of determining vitamin content of various substances by means of the magneto-optic apparatus. This apparatus has been shown to be very sensitive and accurate as well as very rapid in making chemical analyses. It was used to discover the elements 85 and 87 and to determine the isotopes of various substances including those of Hydrogen.
II. HISTORICAL

The absorption spectra of substances containing vitamins have been studied ever since it was known that vitamin D was formed photochemically by ultra-violet light.

Heilbron, Kamm, and Morton (1) studied the absorptive spectrum of cod-liver oil with vitamin D and observed selective absorption in the region 3200A. They also found that cholesterol contained another substance which showed well defined absorption bands at 2930A, 2800A, and 2690A while cholesterol had only general absorption. They concluded that the unknown substance in cholesterol was closely related to the vitamin D precursor.

The absorption band at 3200A was also found by Woodrow (2) who used a photoelectric spectrometer. Woodrow also recorded the three absorption bands of ergosterol in thin films of cod liver oil. His work showed that the absorption band near 2700A-2900A is associated with vitamin D.

Later Morton and Heilbron (3) showed that the absorption band in the neighborhood of 3200A was related to the growth promoting factor, vitamin A. They found that the band at 3230A was present in all liver oils which were
active in promoting growth and that the intensity of this band was related to the antimony trichloride blue color test of Carr and Price (4). The absorption spectra of liver oils and the antimony trichloride color test were compared by Gillam and Morton (5), and they found that the color test was only a rough guide to vitamin A potency.

The antimony trichloride blue color test for vitamin A was criticised by Hawk (6) who found that cod-liver oil exposed to the atmosphere actually gave a deeper blue color than oil which he had kept in the dark. Heilbron, Gillam, and Morton (7) confirmed Hawk's findings and demonstrated that the blue color obtained with ageing was due to a marked increase in the chromogen producing band at 6060A. Norris and Church (8) also confirmed Hawk's earlier work and later Brode and Magill (9) found that it was possible to produce a band at 6080A or 5780A or both by changing the concentration or temperature, or by the addition of certain substances to the oils.

The criticism offered by these various investigators makes it apparent that the antimony trichloride blue color test for vitamin A is not always reliable.

Heilbron, Gillam and Morton (7) compared the ultraviolet absorption band at 3280A with the antimony trichloride color bands in the visible spectrum at 5720A and 6060A and found better correlation between 5720A and 3280A than
between 6060A and 3280A bands. Similar results were obtained by Lovern, Creed, and Morton (10).

Comparisons between the feeding or biological, the colorimetric and spectrographic methods have been made by Coward, Dyer, Morton, and Gaddum (11) who came to the conclusion that the discrepancies between the physical and biological measurements were much greater than the known sampling error in the biological test. They also found that the absorption values at 3280A gave the closest correlation with the biological values, and that fresh oils showed the best agreement between the three tests. Their work showed that with a carefully controlled biological technique the maximum error expected need not be more than 30%. They concluded from their results that, although there is a good correlation between spectrographic and colorimetric methods, the correlation between the physical and biological results is so low that the biological method gives the only absolutely reliable test in determining the vitamin A content of liver oils. These results are in agreement with those of Norris (12) who also conducted a comparative study of the colorimetric, spectrographic, and biological methods for the determination of vitamin A.

Other investigators along this line were Drummond and
Morton (16) who compared the spectrographic method of measuring the vitamin A value of oils with the biological and antimony trichloride tests and found the spectrographic method quite dependable.

A review of the work on vitamin D has been made by Loofbourrow (14). Reerink and Van Wyk (15) have worked with ergosterol and irradiated it. They found that the irradiation of ergosterol produced several substances in succession and that the antirachitic activity was connected with the first stage of the reaction.

The change in optical rotation of ergosterol solutions on irradiation with long ultra-violet rays was measured by Reerink and Van Wyk (16). Their results showed that a linear relationship existed between the change in rotation and the degree of change of ergosterol. From this they concluded that the irradiation of ergosterol gave rise to vitamin D.

The fluorescence of substances containing vitamins has also been investigated. Rosenheim (17) in 1927 noted the fluorescence of ergosterol when irradiated with ultra-violet light. He found, however, that some of the preparations of ergosterol which were not fluorescent became active antirachitically upon irradiation. On the other hand, some fluorescent samples could not be activated.
This led him to conclude that the fluorescence phenomena had no relationship to the formation of vitamin D from ergosterol by ultra-violet light.

Woodrow and Wissink (18)(19) found a fluorescence band in the region 5400A using a mercury vapor lamp for the exciting light and measuring the band by means of a wavelength spectrometer. More work on the fluorescence of vitamin A substances was done by Schmidt (20) who used a Bausch and Lomb quartz spectrograph with a rotating sector photometer. He found that vitamin A substances showed a characteristic fluorescence with a maximum in the region 4600A to 4700A. In addition he found the band at 5400A for animal oils containing vitamin A, but this band was missing for the vegetable oils he tested. He also noted that the intensity of the band was diminished with a reduction of the vitamin A content of the substance.

The chemical structure of vitamin A has been investigated by Karrer, Morf, and Schopp (49), by Bogert (21)(22), and by Heilbron, Morton and Webster (23).

The magneto-optic apparatus which was used in the research work for this thesis had its origin in experiments conducted by Beams and Allison (24) which were designed to measure the time lag in the Faraday Effect. Later Allison (25) used this apparatus to study the time lag as a function of the wavelength of light. The time lag was known to be very
small and so a modification of the method used by Abraham and Lemoine (26), who used the velocity of light as a timing device, was employed.

The magneto-optic apparatus became a new research tool when Allison and Murphy (27) showed that it could be used as a method for chemical analysis. These same men used this apparatus to find the element 67 (virginium) (28). Their work was followed by Allison, Bishop, Sommer, and Christensen (29) and by McGhee and Lawrenz (30) all of whom used the same type of apparatus. Papish and Wainer (31) obtained similar results using a different method.

The isotopes of different metals (32) were also determined by this method as well as the heavy isotope of hydrogen (33). The element 85 (alabamine) was found in sea water and in several other materials by Allison, Bishop, and Sommer (34). Recently McGhee and Lawrenz (52) have found minima for organic substances with the magneto-optic apparatus.

The exact nature of the phenomena underlying the magneto-optic method is unknown. Several people have advanced theories to explain the phenomena. Allison, Christensen, and Waldo (35) have tested several theories experimentally and found that the phenomena can be explained on the basis of a time lag in the Faraday Effect. Other experiments to support this hypothesis were carried on by Allison and
Condon (36). Slack and Breazeale (37) performed several experiments on the magneto-optic rotation by using a condenser discharge and found that the clockwise rotation was greater than that in the opposite direction when the field was reversed.

In the usual procedure of operating the magneto-optic apparatus the eye has been used to detect the minima, but attempts have been made to use a photoelectric cell instead of the eye by Allison, Christensen, and Waldo (38). They used two pliotron tubes to amplify the photoelectric current and employed a circuit as described by Du Bridge (39). They, however, had some trouble controlling the amplifier circuit and found the eye to be much faster than the photoelectric cell.
6 inches by 9 inches, separated by double strength
of the same condensers consisted of 14 sheets of copper
or two separate condensers connected in parallel. One
between two parallel plates. The condenser, O, consisted
out of laboratory. It had a layer of fuse and made by MR. Pinney
these, k, was one desquined and made by MR. Pinney
envelope of the spacers. The high voltage rectifier
series each having 6.5 ohm resistance and a current
As consisted of three exciting resistors connected in
R was a Ward Leonard, type 2044, resistance white
the type used with the General Electric Stamping S-1.
ther tube, k. This transformer, XZ, was similar to
need as a filament heater for the high voltage rectifier.
on the other hand, represents a step-down transformer
former with a secondary voltage of 20,000 volts. This
transformer, type L-2, 110 volt, 60 volt, step-up trans-
deremal is in Fig. 1. It represents a 2,5 kVA.
the apparatus used in this investigation is shown

1. Apparatus

A. Apparatus

III. Experimental

12
glass plates, 9 inches by 12 inches. The other condenser had plates 7 inches by 9 inches and fiber for its dielectric. Both condensers were oil immersed and so arranged that the capacity could be varied. The rotary spark gap, S.G., Fig. 1, is shown on plate 4. It was made up of two aluminum disks about 3 inches in diameter and 1/8 inch thick. These were mounted, by means of brass forks, on a bakelite base. Rubber bands were used as belts. The wheels of this gap were rotated in the same direction and were kept comparatively cool while the apparatus was in operation for any length of time. This prevented arcing between the electrodes.

2. Helices and Cells.

$D_1$ and $D_2$, plate 1, represent two identical helices of No.18 D.C.C. wire of about one hundred turns wound on bakelite cylinders 7.5 inches long and 1.5 outside diameter. These cylinders were supported by wooden blocks as shown in plate 1. $D_1$ remained fixed while $D_2$ could be moved back and forth by means of a long screw. Both helices were mounted on a photometer bench. This scale served as a light path scale. A change of 1 cm. on this scale corresponded to .5 cm.
on the electrical path scale.

The glass cells, plate 1, which held the liquid under observation were placed co-axially inside these helices. Small, semicircular, hard-rubber bushings were fastened to the cylindrical cells by means of wax so that the cells would be at the center of the helices.

These cylindrical glass cells were 16.3 cm. long and 2.2 cm. in diameter. The ends of the cylinders were flared and then ground. Glass windows were fastened to these flared ends by means of picene wax or sodium silicate. The picene wax was used very little because it was soluble in most organic solvents. Short glass tubes were sealed into the cylinders near the flared ends through which the cylinders could be filled with liquid.

Arrangements were made inside the helices so that the cylindrical cells were always midway between the ends of the helices. The wooden holders for the helices had bakelite markers fastened to them so that the helices were always in identically the same position with respect to each other and the light path scale.

3. Optical System.

The light used was the 4358A line of mercury. This
line was obtained by passing a light from a small mercury vapor lamp, shown at the extreme left of plate 3 and in the immediate foreground in plate 2, through a Gaertner, type L230, wavelength spectrometer. The eyepiece of the spectrometer was removed and a slit put in its place. A converging lens was placed between the slit and nicol prism, N₁, so that the slit was located at the principal focus of the lens. Plate 3 shows the mercury vapor arc, the spectrometer, and the lens in place. The lens was supported in the brass tube between the first helix and the spectrometer. This tube also contained the first nicol prism, N₁.

The nicol prisms, N₁ and N₂, were quite large, 16mm. to 20mm. face. The first, N₁, was placed in the brass tube seen in plate 3 between the first helix and the spectrometer; the second nicol, N₂, was placed in tube between the second helix and the photoelectric cell. N₁ served as the analyzer and N₂ as the polarizer. Both nicols were mounted on the photometer bench as shown in plates 1 and 2.

4. Trolleys and Electrical Path Scale.

The trolley system was made up of four sets of wires. The two inside sets, T₁ and T₂, Fig.1, were used for the sliding trolleys while sets T₃ and T₄
were used as auxiliary wires to increase the length of the electrical path. These wires and trolleys were placed overhead and fastened, as shown in plate 4, by means of glass insulators to hooks which in turn were fastened to the wall. The hooks were adjustable so that the tension of the wires could be varied.

The electrical path scale was placed directly above the trolley, $T_2$, as shown in plate 5. It was made of tin and supported at both ends. Turnbuckles were used to support the scale so that it could be held very taut. The smallest division of this scale was 1.5 cm.

The movable trolley, shown in plate 5, was made of bakelite and fiber. The cross pieces were bakelite and the long strip to which the cord was fastened was fiber. The wires passed directly through small holes drilled in the bakelite. Contact was made by means of a brass strip fastened to the center cross-piece. The end cross-pieces served to prevent twisting of the trolley. The trolley was moved along the scale by means of a pulley system. One of these pulleys is shown in plate 5, and the control pulley is shown at the extreme right in plate 3. The trolley was moved either by an electric motor shown in plate 2 or by a hand wheel the shadow of which appears directly over the control pulley in plate 3.
Just above the brass strip of the movable trolley a pointer was fixed which extended up alongside the electrical path scale.

5. Photoelectric Cell Circuit.

The photoelectric cell circuit is shown in Fig. 2. The photocell used was a quartz potassium hydride cell, which according to Ives and Kingsbury (40) is the most sensitive in the region 4300Å to 4400Å.

A modified Du Bridge (39) amplifier circuit was used. The tube shown at the top of the diagram was the FP-54 Eliotron made by the General Electric Company. This tube is a space-charge grid tube designed to have a very high input resistance and a very low d.c. grid current. It is useful either for measuring very small currents, or for measuring voltages in high resistance circuits.

The 90 volt battery which was used as the source of potential for the photoelectric cell consisted of two 45 volt radio "B" batteries. Two standard automobile storage batteries connected in series served as the other sources of current.

The high resistance labelled $10^7$ to $10^{12}$ ohms in Fig. 2 was actually $3.8 \times 10^{10}$ ohms. This resistance
was purchased from the S. S. White Dental Mfg. Co. at New York City. A 20 ohm resistance was placed in the filament circuit of the tube and another 20 ohm resistance was placed as a shunt across the galvanometer.

The whole circuit including storage batteries, photoelectric cell, and piotron tube were all placed in a metal covered box. (See plates 2 and 3.)


The galvanometer used was a Leeds and Northrup HS reflecting type, listed as No. 2285-a in their catalogue. This instrument was mounted directly on top of the metal covered box containing the photoelectric cell and amplifying circuit. A metal shield fully enclosed the galvanometer except for a small opening to permit a beam of light to fall on the mirror. This shield was painted black and consequently does not show very well in plate 2. The metal shield as well as the metal covered box was very carefully grounded.
B. Method of Procedure

1. Preliminary Steps.

In order to calibrate the apparatus the following procedure was adopted. The cylindrical cells were both filled with carbon bisulfide and placed in the helices. The mercury vapor lamp was started and the wavelength spectrometer set at 4358A. The spectrometer, cells, nicols, and lenses were lined up so that the light could pass through them and fall directly on the photoelectric cell. The nicols were set parallel, i.e. uncrossed. This permitted the maximum of light to pass through the system.

The rapid discharges across the condenser, C, Fig. 1, afforded the current surges which gave rise to the magnetic fields in $D_1$ and $D_2$. The broken lines in Fig. 1 represent No. 18, bare copper magnet wires, each 6 meters long. These wires carried the transient current set up by the discharging condenser. The trolleys $T_1, T_2, T_3$, and $T_4$ made electrical connections between the wires. An electric current surge reaching the point $A$ was divided into two parts. One part of the current followed the branch $A T_4 T_1 D_1 B$ while the other part followed the branch $A T_3 T_2 D_2 B$. The two surges of current were made to flow in the same direction around the helices.

Faraday discovered in 1845 that a beam of plane
polarized light, when passed through a transparent, isotropic medium traversed by a magnetic field of force parallel to the direction of the light, suffered a rotation. He also found that the magnitude of the rotation depended upon the strength of the field, the length of the light path in the substance, and the nature of the substance (41)(42).

The light which came from the spectrometer was plane polarized by the nicol, \( N_1 \). Since the nicol prism, \( N_2 \), was parallel with \( N_1 \), all of the light, except that which was absorbed within the cells, could pass through \( N_2 \) and reach the photoelectric cell when there were no current surges flowing through the helices. The presence of rapid current surges in the helices rotated the plane of polarization of the light, and consequently, this affected the amount of light passing through the nicol, \( N_2 \). In order to have the minimum amount of light passing through the nicol, \( N_2 \), it was necessary for the current surges in \( D_1 \) and \( D_2 \) to be so related that the maximum effect would be produced in \( D_2 \) just as the light which had been rotated in \( D_1 \) arrived at \( D_2 \). On the other hand, if these surges were so related that the effective fields acting on the beam of polarized light were in opposite directions, the rotation produced in \( D_2 \) would be equal
and opposite to that in $D_1$ and, consequently, the resulting plane of polarization would be parallel to the original plane so that the beam would pass through the analyzing nicol as if there had been no field present.

2. Finding the Zero Position.

This condition, i.e. the proper relation between the establishing and releasing of the magnetic field in $D_1$ and $D_2$, was obtained by moving the trolley $T_1$ back and forth along the scale until a galvanometer deflection was noticed with $T_2$ placed directly beneath the zero reading on the scale. $T_1$ was used as a movable trolley just to find the zero position; after that $T_1$ became stationary, and $T_2$ was used as the movable trolley.

Electrically speaking, the zero position was found when the electrical length of the path $A T_4 T_1 D_1 B$ was about equal to that of $A T_3 T_2 D_2 B$. The time that was required for a current surge to travel from $A$ through $T_4$ and $T_1$, to $D_1$ was equal to the time for a current surge to travel from $A$ through $T_3$ and $T_2$, to $D_2$ plus the time needed for light to travel from $D_1$ to $D_2$.


In order to check up on the current surges through
the two branches of the circuit, a radio frequency thermo-ammeter was inserted in the circuit: first, between $D_1$ and $B$, and later between $D_2$ and $B$. The current was found to be the same in each branch of the circuit. Tests were also made to determine the variation of the current with a change in the position of the trolleys, but no appreciable variation in current was noted.

4. Reading Minima.

After the zero position had been found, the cylindrical cell, $D_1$, was filled with another liquid. Particular care was taken to clean the cell, $D_1$, thoroughly before the new liquid was added. This was done to remove all traces of carbon bisulfide.

With the new solution in $D_1$ the trolley, $T_2$, was moved very slowly along the scale until another deflection of the galvanometer was noticed indicating a light minimum. The position of the trolley, $T_2$, was noted on the electrical path scale for this minimum. The zero of the electrical path scale was located near the left end of the copper wires, Fig. 1. The trolley, $T_2$, was moved to the right and, consequently, the electrical path, $A T_3 T_2 D_2 B$, was increased. This caused the current surges to reach $D_2$ later than they reached $D_1$. Such
minima have been interpreted as due to a time lag in
the Faraday effect, that is, upon the assumption that
the Faraday effect in the first cell, $D_1$, lagged be-
hind the magnetic field a longer time than it did in
the second cell, $D_2$. The rotation in the first cell
lagged behind the magnetic field so much that it was
necessary for the electrical path, $A T_3 T_2 D_2 B$, to
be longer than $A T_4 T_1 D_1 B$ so that the magnetic field
would be applied later, that is, so that the rotation
effect would be produced in $D_2$ at exactly the time when
the beam of light which had been rotated in $D_1$ reached
$D_2$.

5. Using Light Path Scale.

A similar minimum was obtained for this new liquid
by a different method. $D_2$ was filled with carbon bi-
sulfide and $D_1$ with the new liquid. The trolley, $T_2$,
was placed at the zero position of the electrical path
scale and kept there. Instead of moving the trolley the
cell, $D_2$, was moved away from cell, $D_1$, until a minimum
was obtained. This was done by turning the long screw
which moved the photometer holder and cell, $D_2$, towards
the left, plate 5. In this case the current surges
reached both helices at the same time, but the lag in
the Faraday effect in the liquid in D₁ was greater than in D₂. This difference in the lags was equal to the time it took the light to travel from D₁ to D₂. By changing the position of the trolley, the time at which the surges reached the helices was varied; and by moving the helices, the time at which the light reached the second helix was varied. The scale readings for both procedures checked each other.

The light path scale was comparatively short; therefore, it was used very little. The divisions on the light path scale were twice as large as those on the electrical path scale due to the fact that the current had a return path.

6. Checking Allison's Results.

Several liquids were tried in the apparatus, and it was found that their minima checked with the results of Allison and Murphy (26). Some of the solutions checked were: hydrochloric acid, nitric acid, zinc chloride, barium chloride, water, ferrous sulphate, and ferric chloride. The solutions used were all very dilute. The compounds were all C.P. and were obtained from the Chemistry Department. Table VIII contains the minima found for the different substances that were checked.
The scale readings corresponding to the several minima of these various solutions depended upon the differential time lag in the Faraday effect between the solution in question and carbon bisulfide. For example, the minima characteristic of hydrochloric acid were 15.75 and 15.85. This meant that the differential time lag in the Faraday effect between hydrochloric acid and carbon bisulfide was $15.75 \times 10^{-10}$ sec. and $15.85 \times 10^{-10}$ sec. This was true because it was assumed that the current surges along the wires travelled with the velocity of light, $3 \times 10^{10}$ cm. per sec. Since each division on the electrical path scale was 1.5 cm. and the current had to travel twice that distance, or 3 cm., each division on this scale was interpreted as $1 \times 10^{-10}$ sec.

Experiments were also made on these minima readings with the nicol prisms crossed and the magnetic fields in the helices opposing each other. Minima were found at the same positions of the trolleys as before. This was in agreement with the results of Allison, Christensen, and Waldo (35). The apparatus used in this experiment seemed to work better, however, with the nicols uncrossed and the magnetic fields assisting; therefore, this arrangement was used throughout the
experiment.

7. Adjusting Photoelectric Cell Circuit.

Before any minima could be taken with the apparatus, it was necessary to get the photoelectric cell and amplifier circuit balanced and adjusted. The circuit shown in Fig. 2 was adopted rather than the regular Du Bridge (39) circuit. This was done because the circuit was not used as a current measuring device, but only as a device to detect small changes in current.

The FP-54 tube apparatus, including all wiring, controls, batteries, and photoelectric cell were built in a shielded compartment as shown in plates 1 and 2. Because of the high leakage resistance of the tube any change in the total capacity of the control grid circuit to ground, such as might be caused by stray capacitive effects, would cause a change in potential of the control grid. An appreciable length of time would then be required for the potential to return to its original value. These difficulties were eliminated by complete shielding.

The grid lead and all the connections in the grid circuit were made as directly as possible to avoid the
use of insulators. The connection between the photoelectric cell and the control grid was only three inches long. It had a binding post soldered at its center; one side of the high resistance, $3.8 \times 10^{10}$ ohms, was connected to the binding post. The photoelectric cell was protected from light from the filament of the pliotron tube by a shield placed between the cell and the tube.

Storage batteries of large capacity were used so that the supply voltage varied very little. All of the connections on the storage batteries were soldered. The controls used, such as, rheostats and switches, were of high quality so that positive contact was assured. The storage batteries were kept fully charged.

The surface of the Pliotron was kept clean to prevent surface leakage. Alcohol was used to remove the foreign matter which might form a conducting layer from the control grid to the ground.

The filament resistance of 20 ohms was adjusted so that the voltage across the tube was 2.5 volts. The position of this rheostat was then marked, so that the voltmeter was not needed during the remainder of the experiment.

In order to balance the photoelectric cell and
amplifier circuit the mercury vapor lamp was started and light was allowed to fall on the photoelectric cell. The filament rheostat of the tube was next turned on and the switch between the 10,000 ohm rheostat and the battery closed. As the light from the mercury vapor lamp became constant the shunt resistance on the galvanometer was gradually increased. (This resistance was always zero when the apparatus was not in operation.) The 50,000 ohm and 10,000 ohm rheostats were used to keep the galvanometer deflections at zero. It usually required from one to two hours before it was possible to keep the galvanometer at a zero deflection without any occasional drifts. These drifts were caused by charges which were picked up by the tube leaking off through the control grid circuit. This increased the grid current and caused a drift in the galvanometer. These charges usually leaked off in an hour or two, and the amplifier circuit could be used without trouble. As soon as the circuit had become constant, the shunt resistance on the galvanometer was cut out to give the galvanometer maximum sensitivity.

Whenever a minimum was found the light falling on the photoelectric cell was diminished; and, consequently, the current through the high resistance was decreased,
causing an increase in potential across the high resistance. This rise in potential was impressed on the grid of the tube causing a change in plate current, which in turn caused a deflection of the galvanometer. In the circuit the plate current was balanced against the current through 50,000 and 10,000 ohm resistances; and, since the latter remained constant, any variation in the plate current caused a galvanometer deflection.

A Xylol-Alcohol cell was tried as a high resistance in the circuit, but it was found that the fixed resistance used was far more constant than the cell. Higher resistances than \(3.3 \times 10^{10}\) ohms were also tried, but these slowed down the galvanometer deflection so much that they could not be used. Attempts were made to make high resistances by ruling India ink lines on drawing paper and using these; but good contact was hard to make and these resistances were discarded.


A tray of calcium chloride was placed in the box which shielded the amplifier circuit to keep the air dry. During very humid weather this precaution was not sufficient to keep the circuit in operation. In order to be assured of a dry atmosphere surrounding the Pliotron
and photoelectric cell, a stream of dry air was blown into the box near the bottom and allowed to pass out through the small crevices between the box and the lid. This stream of air was passed through calcium chloride which absorbed the moisture. The tray of calcium chloride was left in the box so that the air would remain dry after it reached the box.
C. RESULTS

1. Explanation of Results.

In order to find whether or not there were distinct minima for vitamin A, the cell, D1, which had a capacity of 46.7 cc., was filled with pure ethyl alcohol, and one drop of Haliver oil was added. (The Haliver oil, which had 100 times the vitamin A potency of ordinary cod-liver oil, was obtained from Parke, Davis and Company, Detroit, Michigan.) The apparatus was then started and minima were read as the movable trolley was moved along the electrical path scale from 0 to 41. Each scale reading which corresponded to a galvanometer deflection was recorded. This procedure was followed five times and the scale readings were then rechecked. Only those scale readings which checked three times in five and which could be rechecked were considered.

After this was done, the tube which contained both the Haliver oil and alcohol was thoroughly cleaned and filled with pure alcohol alone. Five sets of minima readings were then taken and rechecked as before. The two final sets of scale readings were then compared and it was found that there were several scale read-
ings which seemed characteristic of Haliver oil, because they were present in the Haliver oil and alcohol solution but not in the alcohol alone.

These scale readings, or minima, were again checked by putting a drop of Haliver oil in a cell filled with pure water. (The water which was first used was distilled in a block tin still. Later water from a quartz still was used.) The results are shown in table I.

Table I shows that some of the Haliver oil was absorbed by the water, because minima were obtained for the Haliver oil in water which were not obtained for water alone. Moreover, it is interesting to note that the water distilled in the block tin still did show minima which were characteristic of tin. In the case of several readings the galvanometer deflections were weaker when Haliver oil was dissolved in water than when it was dissolved in alcohol. (These faint readings are followed by the letter "f" in the table.) The scale reading, or minimum, at 40.20 did not show in the water solution. This was probably due to the fact that the fraction of the Haliver oil which caused that particular minimum was not soluble enough in water.

In table I are also shown the results obtained by using ordinary Norwegian cod-liver oil. In each
case one drop of oil was added to a cell of solvent. Water and pure ethyl alcohol were again used as solvents. The reading at .89 which was found in Haliver oil was absent in both the alcohol and water solutions of cod-liver oil. The minima: 11.36, 40.20, and 40.45 were all lacking in the water solution of cod-liver oil, whereas in the Haliver oil solution in water only the 40.20 minimum was lacking. The minima in the water solution of cod-liver oil were weaker than those in the alcohol solution, especially in the case of 18.41, 23.38, 25.49, and 34.20.

A sample of Norwegian cod-liver oil was taken and boiled for seven hours. Throughout the boiling air was bubbled through the oil. At the end of the boiling period the cod-liver oil had a distinct brownish color. The boiling of cod-liver oil in the presence of air is known to reduce its vitamin A content. One drop of this oil was added to a cell filled with alcohol and minima were read in the usual way. Also, one drop of this boiled oil was added to a cell full of water and readings were taken. The results of these readings are shown in table II. The water solution had fewer minima than the alcohol solution. Comparing these results with those in table I it will be
noticed that the boiled cod-liver oil had less minima than the ordinary unboiled oil. These results showed that the boiled cod-liver oil had lost some of its strength and that alcohol was a better solvent than water. In the water solution of the boiled cod-liver oil there were four less minima than in the alcohol solution. There was, however, an additional minimum at 26.17, but it was rather faint.

Another sample of Norwegian cod-liver oil was placed in a double boiler and heated for fifteen hours while air was bubbled through it. The temperature of the oil was maintained at 92° ± 3°C. One drop of this oil was added to a cell filled with alcohol and minima which were observed are shown in table II, column 3. This solution was diluted with alcohol and readings were again taken. Four parts of alcohol were used to one part of solution. Several minima which appeared in the undiluted solution did not appear in the diluted solution.

Similar tests were made with spinach juice obtained from a small can of Del Monte spinach. The juice was filtered and added to a cell filled with alcohol. A water solution of spinach juice was also tested. In each case one drop of the filtered spin-
ach juice was added to the cell filled with the solvent. More minima appeared in the water solution than in the alcohol solution.

The juice from a California Sunkist orange was also tried in both water and alcohol solutions. Here, as before, one drop of the filtered juice was added to a cell filled with liquid, and the minima read.

The minima for the spinach juice and orange juice are given in table III. Here again the water solutions of the two juices gave more minima than the alcohol solutions.

Table IV contains the minima read on carotene and tomato juice. (The carotene solution was obtained from Mead, Johnson and Co., Evansville, Ind.) It had 10 times the antixerophthalmic potency of ordinary cod-liver oil. One drop of this solution was added to a cell filled with alcohol and another to a cell filled with water. Minima were read and recorded as shown in the table. A ripe tomato was the source of the tomato juice which was used. One drop of the filtered tomato juice was used and minima were read as in the preceding cases. The minima at 18.41, 40.20, and 40.45 were lacking in the tomato juice. For carotene the 22.30 minimum as well as the 32.90 minimum
were lacking in both the alcohol and water solutions. This was the first solution which failed to give the minimum at 32.90.

Peanut oil was added to the separate cells filled with alcohol and water, and it was found that this oil showed very few minima. Peanut oil is known to be lacking in vitamin A content. In the case of this oil the minima at .89, 8.94, 25.51, 32.90, 34.20, 40.20, and 40.45 were absent. The results are shown in the first two columns of table V.

The irradiated cod-liver oil was obtained by irradiating ordinary Norwegian cod-liver oil under a quartz mercury vapor lamp for four and one-half hours, the oil being placed twelve inches from the lamp. A very thin layer of oil was exposed to the ultra-violet light so that the light could penetrate the oil more easily. Since ultra-violet light is known to destroy the vitamin A potency of cod-liver oil, this was done to see how the minima of an irradiated oil would compare with that of a non-irradiated oil. The results obtained with this irradiated oil are shown in the last two columns in table V. In the first determination of the minima one drop of this oil was added to
a cell filled with alcohol, while in the second determination the solution was diluted by adding four parts of alcohol to one part of the first solution. The minima at 18.41 and 34.20 did not appear in the dilute solution. In both cases, however, the minimum at 32.90 was present though very weak.

A carotene solution dissolved in Wesson oil was obtained from Dr. Swanson of the Home Economics Department, who had obtained it from the U.S. Department of Agriculture, Bureau of Chemistry and Soils. The carotene-Wesson oil solution was sealed in a glass tube in an atmosphere of nitrogen so that no oxidation could occur. The solution was kept at a temperature of about 0°C. One drop of this carotene solution was added to a cell filled with alcohol, and the minima read. Table VI shows the results obtained from this solution and also those obtained when the first solution was diluted with alcohol, by using three parts of alcohol to one part of the solution.

The results obtained with carotene crystals and with Wesson oil are also shown in table VI. (The carotene crystals, as well as the carotene solution, were obtained from Mead, Johnson and Company, Evansville, Ind. The Wesson oil was obtained from the Home Econo-
About 20 mg. of carotene crystals were dissolved in 100 cc. of alcohol. This solution was put in one of the cells, and minima read. The carotene crystals dissolved in alcohol produced less minima than the carotene in the Wesson oil solution prepared by the U. S. Department of Agriculture. The minimum at 32.92 was not found in the pure carotene crystal solution nor in the plain Wesson oil solution, but it was found in the carotene-Wesson oil solution which was obtained from the U. S. Department of Agriculture.

In table VII are shown the results obtained when the carotene-Wesson oil solution was made more dilute. In this case six parts of alcohol were used with one part of the original solution. Several minima which were present in the dilute were absent in the very dilute solution; other minima were found in the very dilute solution which were absent in the dilute solution.

Spectroscopic investigations on egg yolks have been carried on by von Euler and Klussman (49). They found the absorption bands characteristic of vitamin A. A sample of egg yolk, obtained from the Poultry Husbandry Department, was tested in this apparatus. This sample was part of the solution used in feeding experi-
ments and was known to contain vitamin A. One drop of the standard egg yolk solution was added to 150cc. of pure water. The minima recorded for this solution included all those found for Haliver oil except the minima at .89, 8.94, 18.41, and 40.45.

An old sample of cod-liver oil which had been 100 times as potent in vitamin A content as ordinary cod-liver oil was found and tried in the apparatus. Not only was this sample of oil very old, but it had been exposed to the air for a long time. It was discolored and very gummy. One drop of it was added to a cell filled with alcohol. The data taken are shown in column 3 of table VII.

Some of the solution, obtained when 20 mg. of carotene crystals were added to 100cc. of alcohol, was exposed to ultra-violet light. This was done by placing the solution under a quartz mercury vapor lamp and irradiating it for four and one-half hours at a distance of 12 inches. Irradiating the carotene solution caused the minima at 8.94, 11.36, 18.41, 23.38, and 32.92 to appear, as shown in column 4 of table VII.

2. Tables. (See following pages.)
**TABLE I**

<table>
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<tr>
<th>Minima in alcohol and Haliver oil but not in alcohol alone.</th>
<th>Minima in water and Haliver oil but not in water alone.</th>
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TABLE V

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IV. DISCUSSION

The minima found with Haliver oil and with cod-liver oil solutions, as shown in table I, show that cod-liver oil has nearly the same minima as Haliver oil. Haliver oil was taken as a standard because it is known to be very potent in both vitamin A and D content. The water solutions of these oils which contained vitamins had fewer minima than the alcohol solutions. The vegetable and fruit juices which were tested were found to have more minima when dissolved in water than when dissolved in alcohol.

The minimum at 32.90 was found for all the substances which were known to contain vitamin A. In the case of the boiled cod-liver oil in a water solution this minimum did not appear, although it was prominent for the water solution of the unboiled oil of the same concentration. Also, the minimum for a weak alcohol solution of cod-liver oil disappeared when the oil was oxidized by bubbling air through it at a temperature of about 92°C. This minimum was likewise absent in peanut oil, in Wesson oil, and in carotene solutions. With irradiated cod-liver oil this minimum appeared only faintly.

Since the minimum at 32.90 was absent in substances which did not contain vitamin A and present in those
substances which did, it was concluded that this minimum is very closely associated with vitamin A. The fact that this minimum was weakened or destroyed by oxidizing, irradiating, or boiling the cod-liver oil showed that it was closely related to the vitamin A content of the oil. Other minima were also examined, but the minimum at 32.90 was the only one for which the evidence was sufficient to indicate a close correlation with vitamin A.

This minimum at 32.90 was not found in any of the carotene solutions except the one carotene-Wesson oil solution. The pure carotene crystals and the carotene solution obtained from Parke, Davis and Company showed no minimum at this point.

Much work has been done in recent years to find the relationship of carotene to vitamin A. Moore (43) (44) found that carrot roots were rich in vitamin A content and that "carotene behaves in vivo as a precursor of vitamin A." Von Euler, von Euler and Hellstrom (51) used the antimony trichloride test on carotenoids and found that they gave a reaction similar to that due to vitamin A, and that carotene from carrots acted the same upon the growth of their test animals as vitamin A.

Duliere, Morton and Drummond (50) questioned the conclusions of Moore and von Euler who said that carotene
could replace the vitamin A in the diet of animals. They
decided after some investigation that the carotene used by
Moore and von Euler was impure and that the impurities
were responsible for the growth promoting factor found
in their carotene. They, Duliere, Morton and Drummond,
also reported that they had found evidence that the growth
promoting factor of liver oils was not due to carotene.

Bowden and Snow (45) irradiated a solution of caro-
tene in cyclohexane in an atmosphere of nitrogen. Their
exciting light was the 2650A line of mercury. After a
few hours of irradiation they found that the solution had
a strong absorption band at 3280A. The irradiated solu-
tion also gave a blue coloration when tested with anti-
mony trichloride in chloroform. From these tests they
concluded that carotene was changed to vitamin A by irra-
diation with ultra-violet light.

The work of Bowden and Snow was criticised by Heil-
bron and Morton (46), who maintained that the experiment
carried on by these men if executed properly would yield
only hydrocarbons.

Recently Dann (47) has found that the rate at which
vitamin A was formed by irradiation with ultra-violet
light from carotene was slower than the rate at which
the vitamin A content was destroyed. Consequently, he
maintained that it would therefore be impossible for vitamin A to be formed when carotene is exposed to ultra-violet light.

In our experiments, carotene in an alcohol solution was irradiated by ultra-violet light; and it was found that after irradiation this solution gave the minimum at 32.90; whereas, before irradiation this minimum was not present.

The results obtained in this investigation so far on vitamin A have shown that the magneto-optic apparatus is very sensitive, and this fact will undoubtedly be very useful in obtaining further information about the physical properties of vitamins. It is planned to continue the investigations and to determine similar minima for other vitamins. It would be possible also with this apparatus, as changed and improved in this investigation, to follow the changes in the vitamins under varying physical conditions. Furthermore, it is hoped that it will be possible to carry on investigations into the nature of the lag in the Faraday Effect.

In this work galvanometer deflections caused by light minima were read on a scale located on the wall of the room. The galvanometer deflections were small and difficult to read. An amplifier tube placed between the
Fliotron and the galvanometer in the amplifier circuit would increase the deflections so that they could be read more easily.

Suggestions have also been made to equip the magneto-optic apparatus with a recording device so that the minima at different scale readings would be automatically recorded. A device of this kind would eliminate the personal element in the reading of minima and facilitate the operation of the apparatus. Such a piece of equipment, properly designed, should be capable of recording accurately on a moving photographic paper the presence of very small quantities of chemicals in any mixture which is transparent to light.

This apparatus should be calibrated for several regions of the spectrum so that solutions of any color could be tested. In this investigation only the blue mercury line at 4358A was used. This caused some trouble because of absorption by some of the solutions investigated. This line was adopted for this work as most of the data available were taken with light of approximately this wavelength.

It has been planned to change the oscillatory circuit of this apparatus. Instead of using the spark gap and the condenser a vacuum tube oscillator will be used
to furnish the current surges through the helices. The vacuum tube circuit would be more constant than the spark gap and would supply current surges of the same amplitude.
V. CONCLUSIONS

The conclusions which may be drawn from the results of this investigation are as follows:

1. The photoelectric cell may be used successfully with the magneto-optic apparatus.

2. The magneto-optic apparatus can be used to determine the vitamin content of various substances.

3. The minimum at 32.90 on the light path scale used shows evidence of being closely related to vitamin A content of different substances, and it is very probable that this minimum at 32.90 is characteristic of this vitamin.

4. Carotene in an alcohol solution after being irradiated by ultra-violet light gives the minimum at 32.90.

5. Pure carotene in an alcohol or water solution does not give the minimum at 32.90.

6. The magneto-optic method is very rapid in determining the vitamin content of different substances.
VI. SUMMARY

In this investigation the magneto-optic apparatus was used. This apparatus was modified to some extent from the original apparatus used by Allison. The modifications included a mercury vapor arc and a monochromator to furnish a steady source of monochromatic light, as well as a photoelectric cell circuit and amplifier to read the minima. Only half of the bilateral trolley system used by Allison was employed.

The apparatus gave evidence of a distinct minimum which seemed characteristic of vitamin A. This minimum was located at 32.90 on the light path scale. Pure carotene did not give this minimum. Pure carotene irradiated with ultra-violet light, however, did give this minimum.

Plans for the extension of the work on vitamins have been made with the view in mind of finding minima characteristic of the other vitamins. Plans for improving the operation and facilitating the work of the apparatus have also been made.
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VIII. ACKNOWLEDGEMENTS

The author wishes to acknowledge his indebtedness to Dr. Jay W. Woodrow, Head of the Department of Physics, for furnishing the necessary apparatus and supplies to carry on this research and for his untiring assistance in answering questions and giving valuable suggestions throughout this investigation.
Fig. 1 Magneto-Optic Apparatus.
Fig 2
Amplifier Circuit.
PLATE 1

A close-up of the helices and of the brass tube containing the analyzing nicol and the first lens is shown. At the bottom of the plate may be seen one of the cylindrical cells with the flared ends and the windows fastened to the ends. The small, vertical tube located at the left end of the cylinder served as an opening through which liquids were put in the cell. The pointer on the photometer holder was colored white so that it would appear in the photograph.
PLATE 2

This plate shows the arrangement of the apparatus as viewed from the left. The mercury vapor lamp was not in place because it obstructed the view of the helices. The metal covered box enclosing all of the photocell and amplifier circuit is shown at the extreme left. The shielded galvanometer, mounted on the corner of this box, is barely visible because the shield was painted a dull black. A small, reversible d.c. motor used to operate the movable trolley may be seen in the lower left-hand corner. The long screw used to move the second helix back and forth along the scale is easily recognized.
Plate 3 shows the right-hand side of the apparatus. In this plate the mercury vapor lamp is in place in front of the slit of the spectrometer. Another view of the screw used to operate the movable helix is shown. The lens and the first nicol prism are located between the spectrometer and the first helix. The control panel of the amplifier and photoelectric cell circuit, located on the front of the metal box, is visible. The switches used to operate the spark gap, the high voltage transformer, the mercury vapor arc, and the lights to illuminate the scale are found at the right.
PLATE 4

This plate shows the spark gap used. The aluminum disks were rotated by means of a small electric motor. The speed of the disks was regulated by means of the screw which controlled the friction drive on the motor. During operation the motor and the spark gap were mounted inside the black, metal box shown in the background. This box prevented the light from the spark gap from illuminating the room. Glass and bakelite insulators were used for the high tension leads to the spark gap so that no short circuit was made through the box. Rubber bands were used as belts.
The movable trolley is shown here as well as the electrical path scale. A small section of the trolley system is shown. The glass insulators used and the hooks with which these were fastened to the wall are clearly visible. One of the pulleys and the cord used to operate the movable trolley are seen in the lower right-hand corner. A similar pulley was located at the other end of the wires; and a larger pulley and control wheel were located near the control switches shown in plate 2. This pulley system afforded the control over the movable trolley.