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The Application of a Photronic Nephelometer in the Determination of Calcium

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The Application of a Photronic Nephelometer in the Determination of Calcium

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VITA

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A survey of the literature on photronic nephelometry has disclosed few papers on the subject. Canals and Hotala showed that in nephelometry in transmitted light the photoelectric cell is far superior to the eye.[1] Greene describes a photronic nephelometer and gives data on the rate of development of opalescence of AgCl dilute solutions under various conditions to show the degree of reproducibility that can be attained with this instrument.[2]

This paper describes a type of photronic nephelometer that was constructed from materials that many laboratories have on hand and demonstrates one of its applications, the rapid determination of small amounts of calcium.

The instrument was so designed that it would be possible to run many samples in a short time. To do this it was found convenient to use oil sample bottles with optically plane bottoms as containers for the suspensions. These can be easily lifted into or out of the nephelometer.

As a support and a shield for the bottles a brass cylinder of sufficient diameter to admit the bottles was attached to a brass plate that covers the bottom of a 15" by 8" water bath. In figure 1. the oil sample bottle is shown standing

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1 Canals, E. & Hortala, A., Bull. soc. chim. biol. 15, 1535-1551 (1933)

2 Greene, C. H., J. Am. Chem. Soc., 56, 1269-72 (1934)

beside the shield that it fits into when the nephelometer is in use. There is an opening in the plate where the cylinder is attached so that the light scattered downward by the suspension can reach the photronic cell that is directly below the opening under the cylinder. The cylinder has a 1" by 3" aperture on one side that permits the beam of light to shine on the enclosed suspension. The photronic cell is held in place by a little light-tight box that is attached to the under side of the wooden base that supports the water bath. The openings in the base and in the brass plate are the same size as that on the photronic cell. A photographic shutter of the double blade type pneumatically operated covers the opening on the wooden base so that by manipulating the usual rubber bulb one can shield or expose the photronic cell as one desires. The brass cylinder and the plate are coated with black paint to prevent stray beams of light from being reflected into the system.

Other apparati used for the photronic nephelometer are: a Leeds and Northrup Galvanometer type R resistance 570 Ohms complete with scale, a Weston Photronic Cell, and parts of a projection lantern. (See arrangement of instruments in figure 2.) To prevent the beam of light from the projection lantern from warming up the water bath a heat filter is placed so that the beam of light passes through it before the light enters the water bath. A glass bowl having two paral-

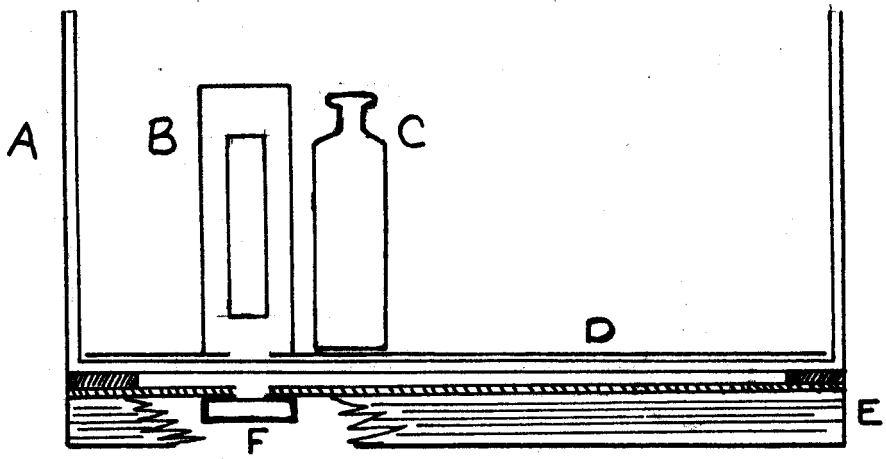

Figure 1. Water bath and photronic cell of nephelometer.

A. Water bath
B. Support and shield for bottle C.
D. Copper plate
E. Wooden base
F. Photronic cell.

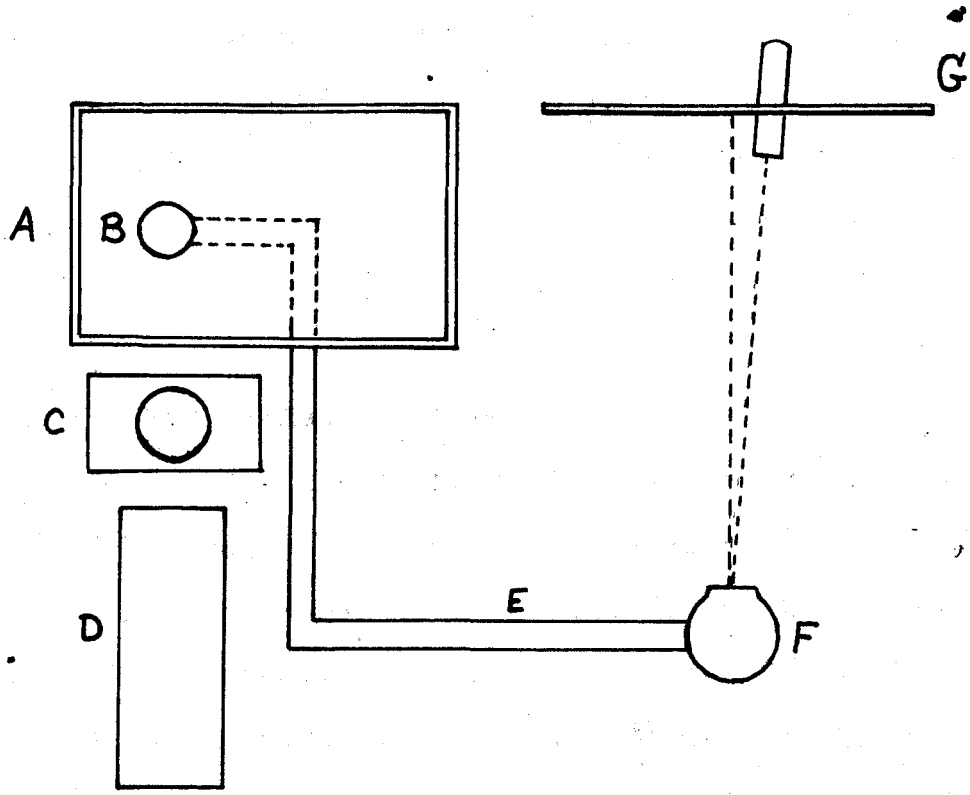

Figure 2. Arrangement of apparatus and partial wiring diagram.

A. Water bath
B. Photronic cell
C. Heat filter
D. Projection lantern
E. Wire connections from photronic cell to galvanometer
F. Galvanometer
G. Galvanometer scale.

lel sides and containing a copper sulfate solution was the heat filter used. To insure a constant illumination from day to day all of the measurements with the nephelometer were made in a dark room.

REAGENTS

AMMONIUM STEARATE REAGENT - Directions: Dissolve 4.0 grams stearic acid and 0.5 ml. oleic acid in 400 ml. of hot alcohol (95%). Add 20 grams ammonium carbonate dissolved in 100 ml. of hot water. Allow the solution to boil for a minute or so, cool, add 400 ml. of alcohol, 100 ml. of water and 2 ml. of concentrated ammonium hydroxide. Filter. The solution should be water clear and colorless.

STANDARD CALCIUM OXALATE SOLUTION - Directions: Dissolve 72.9 mg. pure $CaC_2O_4.H_2O$ in 25 ml. of 2N. HNO_3 and dilute to 1000 ml. with water. Mix well. This solution contains 0.02 mg. of Calcium per ml. and is 0.05 N in HNO_3.

HEAT FILTER SOLUTION - Directions: Dissolve 0.5 gram of copper sulfate dissolved in 100 ml. of water and add 1 drop of concentrated sulphuric acid. This solution is stable for two weeks.

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[1] Yoe, John H. Photometric Chemical Analysis Vol. II Nephelometry. John Wiley and Sons, New York (1929)

[2] Lyman, H. J. Biol. Chem. 29, 169 (1917)

PROCEDURE

To insure a degree of uniformity in preparing suspensions this procedure was followed:

Add distilled water to the measured calcium solution to make a volume of 75 ml. Then while steadily stirring add 50 ml. of ammonium stearate solution at the rate of 50 ml. in three minutes in each case. This can be done by using a burette and running in the solution at the rate of 1 ml. in three seconds. Transfer the suspensions to the optically plane bottomed bottles, using no wash water. Let stand for fifteen minutes in the water bath of the nephelometer at 25°C. The samples are then ready to be measured.

In using the photronic nephelometer, which in these experiments was in a dark room, the water bath is set at 25°C. The galvanometer is adjusted to zero reading with the scale set at the distance of 1 meter from the galvanometer. The shutter covering the photronic cell is closed, and the beam of light from the projection lantern is directed through the heat filter upon the open side of the cylinder containing the suspension to be measured.

In order to have a broader parallel beam of light the entire lens system except one condenser was removed from the projection lantern. After the galvanometer is adjusted to zero, the shutter is opened exposing the photronic cell to the light

that is being scattered downward by the suspension. The deflection in millimeters can then be read on the galvanometer scale.

RESULTS

First six suspensions all containing 0.5 mg. of calcium in a volume of 125 ml. were prepared and their deflection was measured on the galvanometer of the nephelometer. The results are tabulated in Table I. From this preliminary experiment it was decided that this photronic nephelometer responded satisfactorily and measured the quantity of light that is scattered downward by the suspensions. It is possible to prepare six calcium suspensions that give a good degree of reproducibility in the development of opalescence, which is one of the most important conditions in accurate nephelometric work.

Readings are given Table II for other suspensions containing amounts of calcium ranging from 0.125 mg. up to 1.5 mg. all in a volume of 125 ml. of solution. These readings are plotted in Graph I. It is interesting to notice that in the case of suspensions containing 0.27 mg. and 0.29 mg. of calcium in a volume of 125 ml. the nephelometer gave a good clear-cut average deflection of 12.25 and 13.25 mm. respectively when compared with the deflection of 11.25 mm. for 0.25 mg. of calcium.

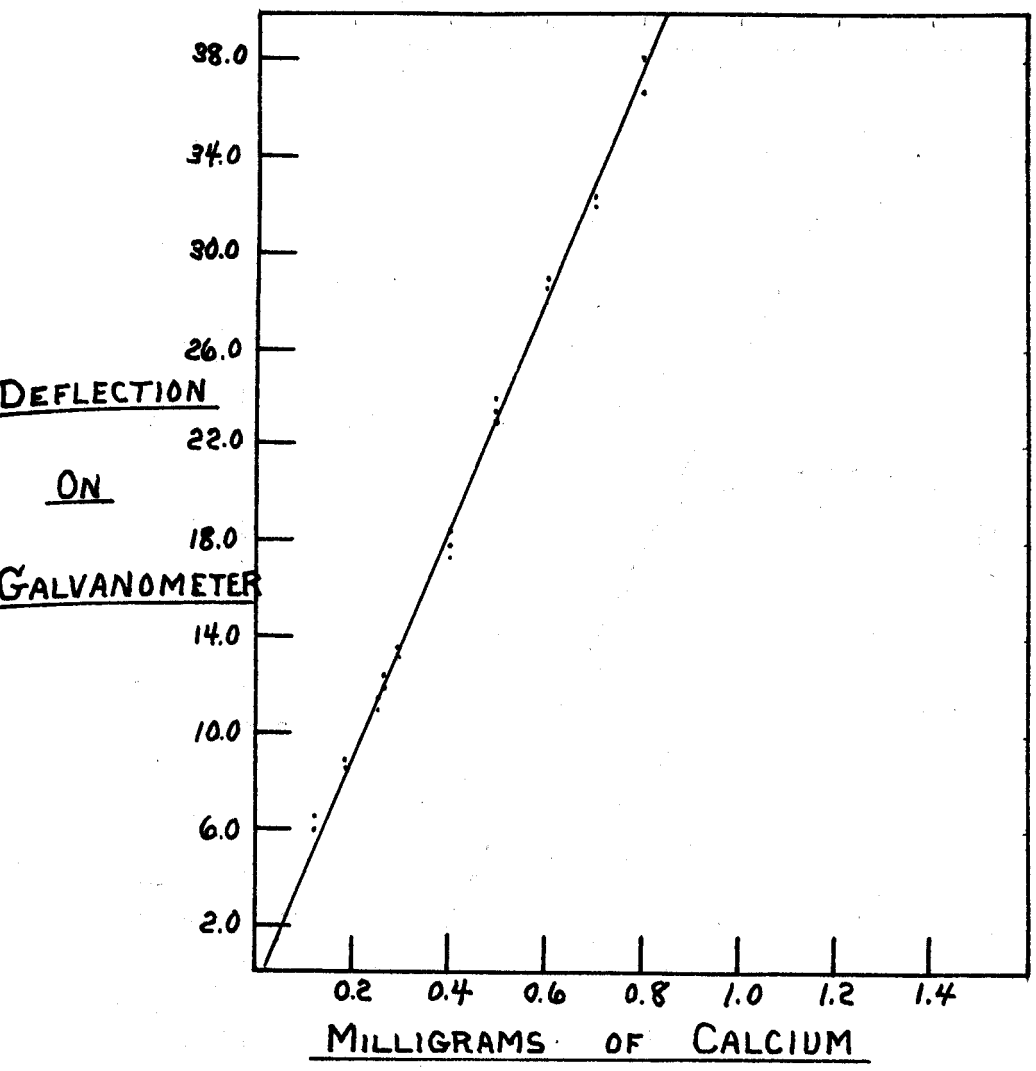
Table I

Amount of Calcium	Deflection in Millimeters	Average Deflection	Average Per Cent Deviation from Mean
0.5 mg.	23.0		
0.5	24.0		
0.5	23.5	23.3	1.5
0.5	23.0		
0.5	23.5		
0.5	23.0		

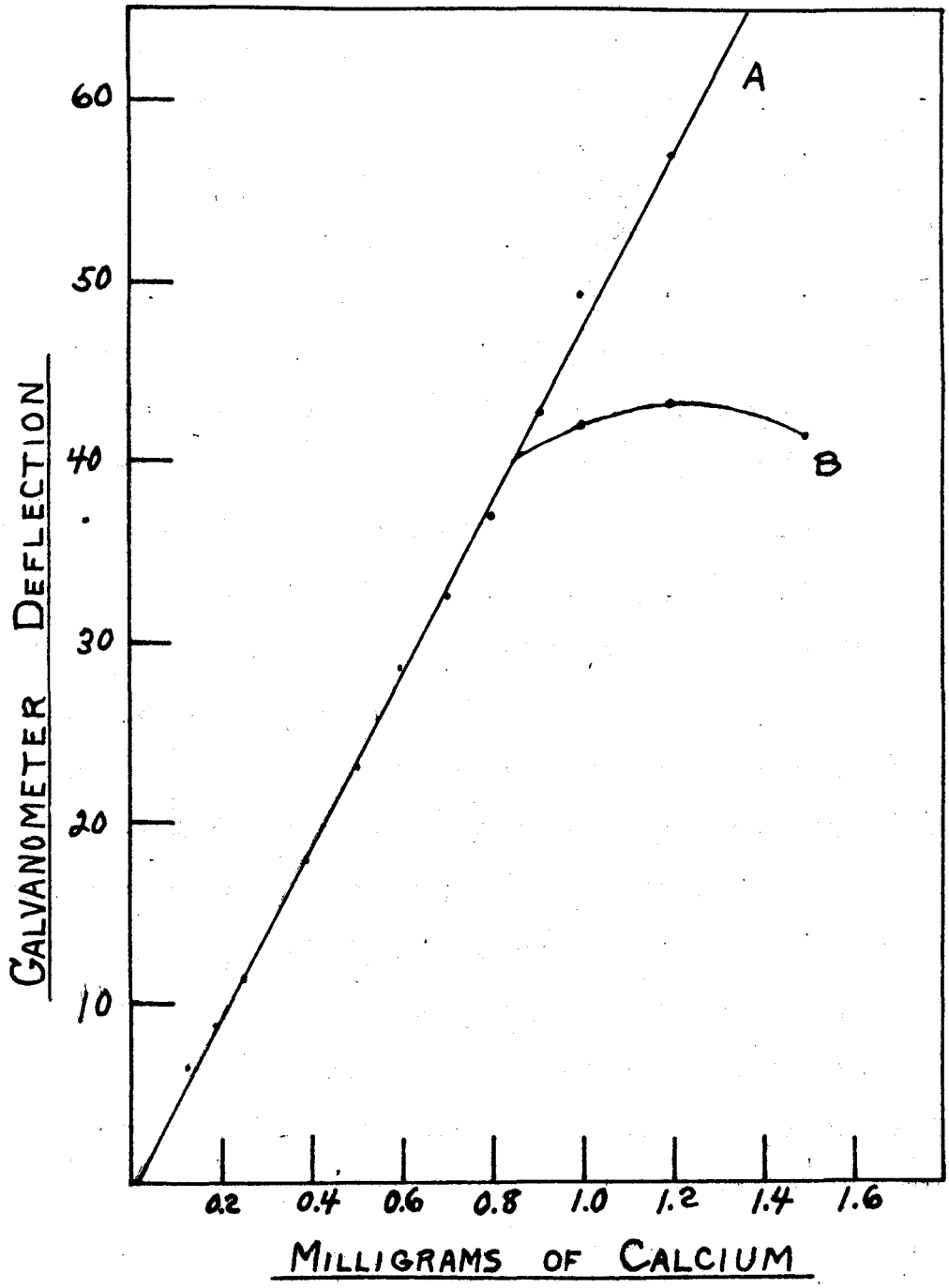
Table II

Amount of Calcium	Deflection in Millimeters	Average Deflection	Average Per Cent Deviation from Mean
0.125 mg.	6.7 6.3	6.5	3.0
0.188	8.6 9.0	8.8	2.3
0.25	11.5 11.5 11.0 11.0	11.25	2.2
0.27	12.0 12.5	12.25	2.0
0.29	13.0 13.5	13.25	1.9
0.40	18.5 18.0 17.5	18.0	1.8
0.50	24.0 23.5 23.0 23.0	23.4	1.6
0.60	29.0 28.5 28.0	28.5	1.2
0.70	33.0 32.5 32.0	32.5	1.1
0.80	38.0 37.5 36.5	37.3	1.5
0.90	43.5 42.0	42.8	1.7
1.00	50.0 48.0	49.0	2.0
1.20	58.0 55.5	56.8	2.1

GRAPH I


38.0
34.0
30.0
26.0
22.0
18.0
14.0
10.0
6.0
2.0
DEFLECTION ON GALVANOMETER
0.2
0.4
0.6
0.8
1.0
1.2
1.4
MILLIGRAMS OF CALCIUM

GRAPH II


A
B
60
50
40
30
20
10
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
GALVANOMETER DEFLECTION
MILLIGRAMS OF CALCIUM

* Following the procedure outlined in this article it was noticed that when calcium was present in greater amounts than 0.8 mg. the curve showed a tendency to reach a maximum and then fall off. See curve B, Graph II. It was decided that possibly there was not enough ammonium stearate reagent present to give a complete reaction with the greater amounts of calcium. The ammonium stearate was then increased to 75 ml. for the 0.8 mg. and higher samples of calcium. The total volume was kept at 125 ml. by using a calcium standard with 1 ml. equivalent to 0.04 mg. of calcium instead of 0.02 mg. The increased ammonium stearate gave readings for the 0.9 mg., 1.0 mg. and 1.2 mg. points that fall in line with the other points on the curve, showing that Beer's law holds for these suspensions. See curve A, Graph II.

Once the calcium curve for the particular instrument that one is using is determined it is not necessary to prepare fresh standards for each comparison. The deflection is determined for the sample, and then by consulting the curve the amount of calcium is known. When new reagents are prepared new standards should be checked against a few points on the curve.

In running unknowns the samples can be taken so that they are of different calcium content. For example, 1 ml., 2 ml. and 5 ml. samples can be taken, and when their readings are compared with the calibration curve, the amount of calcium pres-

ent should be known with fair accuracy. Otherwise, if two or three similar samples are run, they will check with themselves, but in this case it may happen that these readings may fall on the maximum part of the curve, making the results questionable until a more dilute sample is run as a check.

If two suspensions of the same substance containing the same amount of dispersed material are compared in the nephelometer, the readings will be the same only when the particles have the same size. If this condition is not fulfilled, the suspension with the largest particles will show the greatest amount of scattered light or a higher galvanometer deflection.

DISCUSSION

According to Lyman[1] the large excess of stearate in proportion to the calcium to be precipitated is to be noticed. If this excess is not present, the calcium soap, instead of remaining suspended in an even cloud, settles rapidly to the bottom either as a flocculent mass or as a crystaline precipitate, and is, of course, worthless for nephelometric determination.

Koltoff and Sandell[2] mention the following conditions which must be controlled within narrow limits to prepare a precipitate of uniform physical character:

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[1] Lyman, H. J. Biol. Chem. 29, 169 (1917)

[2] Koltoff and Sandell, Textbook of Quantitative Inorganic Analysis. MacMillan and Co. (1937)

1- The concentration of the two ions which combine to produce the precipitate.

2- The ratio of the concentration in the solutions mixed.

3- The manner of mixing.

4- The rate of mixing.

5- The time required to produce maximum scattering.

6- The stability of dispersion

7- The temperature.

8- The presence of other electrolytes.

9- The presence of non-electrolytes.

The solubility of most inorganic precipitates is decreased by the addition of organic solvents immiscible with water. Since the degree of supersaturation then increases the number of particles formed increases also, and the amount of light scattered or absorbed decreases in the presence of organic solvents. The latter, however, stabilize the suspensions.

10- If the suspensions settle too rapidly, more stable ones are frequently obtained by precipitation in the presence of a protective colloid. Again the particle size will be found to be entirely different when the suspension is prepared in the absence rather than in the presence of protective colloids.

Among the applications of turbidity measurements P. V. Wells[1] states that turbidimetry takes its place beside colorimetry as an extremely sensitive method of volumetric chemical

[1] Wells, P. V., Chem. Reviews 3, 376 (1927)

analysis. In grading the size of particles of pigment turbidity is a direct statistical measure much more readily determined than the laborious methods of the microscope and the ultramicroscope.

Mecklenburg[2] showed how particle size affects tyndall intensity. Here is given a tabulation of some of his results:

d cm x 10^{-5}	I
0.05	0.05
0.10	0.88
0.20	6.00
0.30	13.60
0.42	143.50
0.93	180.00
2.46	190.00
8.40	383.00

These results are in accord with Lord Rayleigh's law that for very small particles the Tyndallmeter reading increases proportionally to the cube of the particle diameter.

Tolman, Gerke, Brooks, Herman, Mulliken and De W. Smyth[3] carried out a similar experiment with larger particles and found the intensity of a Tyndall beam decreases with larger size particles. Below are some of their findings:

d cm x 10^{-5}	T
9.97	175
22.4	134
62.4	44
93.9	35
206.0	14

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[2] Mecklenburg, Kolloid -Z 16, 97 (1915)

[3] Tolman, et al., J. Am. Chem. Soc. 41, 575 (1919)

Bechhold and Hebber[1] investigated the nephelometer effect of colloid systems of different particle size with $BaSO_4$ sols. using a Kleinmann nephelometer and found that with the increasing size of particles the turbidity increases rapidly up to particles 800μμ in size; it then falls off more rapidly, then more slowly. Rayleigh's law

$$J = (n v^2 / \lambda) k$$

in which J is the intensity of the scattered light, n the number of particles per unit volume, λ the wave length of the light, and k a constant, holds only for the region of size below 800μμ. From the relation between turbidity and the size of the particles we have a method of measuring the size of amicrons and submicrons provided a standard solution of known dispersity is available for comparison.

Hoffmann[2] determined the average degree of dispersion in suspensions by means of the photo-cell. This method is based on the fact that light absorption in suspensions of particles 2 -30μ in size proceeds in accordance with the formula

$$J = J_0 e^{-Kc/r}$$

where c is the concentration, r the radius of the particles, J the intensity of the scattered light, and J_0 the intensity of the incident light.

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1 Bechhold and Hebber, Kolloid -Z 31, 70-4 (1922)

2 Hoffmann, E. Kolloid -Z 77, 286-8 (1936)

SUGGESTIONS FOR FUTURE WORK

The beam of light could be operated from a source of electricity that would furnish a more constant voltage. Because the intensity of a tungsten filament lamp is given by the equation

$$I = k v^{3.6}$$

any slight voltage change will have its effect on the results.

Readings could be taken keeping calcium constant and varying the ammonium stearate. From these results it is possible that a relationship for determining particle size could be developed.

The instrument could be altered so that smaller vessels for the suspensions could be used. This would reduce the amount of water in the suspension and might cause the ammonium stearate to be more effective.

It is possible that by using a more sensitive galvanometer than was employed in these experiments and by more careful control of experimental conditions that the per cent of deviation may be cut down considerably.

SUMMARY

A photronic nephelometer of easy construction has been described, and its application in the determination of amounts of

calcium ranging from one part per million to one part per hundred thousand has been shown. The deviation varied from 1.1% to 3.0% from the mean in these experiments. The instrument was found to be most sensitive and the curve more accurate when samples containing approximately 0.50 mg. to 0.80 mg. were used.

This instrument should be of real value in this work and in similar determinations provided that its limitations are taken into account.

I wish to express my appreciation for the guidance so graciously and generously given me by Professor G. M. Schmeing.

The thesis, "The Application of a Photronic Nephelometer in the Determination of Calcium," written by Anthony M. Gross, has been accepted by the Graduate School of Loyola University with reference to form, and by the readers whose names appear below, with reference to content. It is, therefore, accepted in partial fulfillment of the requirements for the degree of Master of Science.

Dr. Avdith B. Davist November, 1938

Dr. George M. Schmeing November, 1938