

# A PHOTOELECTRIC NEPHELOMETER FOR CHEMICAL ANALYSIS

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## *Introduction*

CHEMICAL analysis of substances by measurement of the amount of light (i) absorbed, in case of dissolved substances and (ii) reflected, in case of suspended ones, has assumed considerable importance in analytic procedures in recent years. In a previous communication<sup>2</sup> from these laboratories the authors described a new instrument for chemical analytic purposes by measurement of the absorbed light. The present contribution deals with a physical instrument and an outline of the procedure for the analysis of suspended substances by measurement of the reflected light.

The chief advantages of the nephelometers, *i.e.*, instruments for chemical analysis of suspended substances by measurement of reflected light, are their convenience of operation, the large number of analyses that can be done at a time and the possibility of measuring with their aid concentrations which are measurable only with great difficulty, if at all, by gravimetric methods. However, the nephelometers do suffer from certain inherent limitations which need elimination. The dependence on the ability of the eye in the matching of light introduces a personal error in the estimations. Moreover, colour blindness, a defect not uncommonly met, dispenses with the use of the visual nephelometers. The two types (i) "constant upper end type" and (2) "constant lower end type" to which the nephelometers conform, differ in the distance that the light from the longer of the two illuminated and measured columns has to travel from the particles to the eye-piece—greater and smaller (respectively) than that from the shorter column. This variation in the distance factor, must necessarily introduce a certain amount of inaccuracy. Further, any arrangement, dispensing with the use of the plunger, which is likely to disturb the size or dispersion of the suspended particles, is desirable.

The frequent necessity of making a large number of quantitative estimations of soil and plant constituents, by the production of insoluble precipitates, has repeatedly called for simplifying the procedure so as to eliminate

the possibilities of personal error in observations, to detect more minute differences than is possible by the preceding methods and to be applicable for varied purposes. The new instrument described here circumvents some of the defects of the visual nephelometer and allows of certain additional advantages.

### *Principle*

A turbid fluid possesses the property of causing a part of the light illuminating it to be deviated from its original direction and be diffused. The brightness of the light reflected, *i.e.*, opacity, is due to small particles in suspension in the liquid. The intensity of light reflected depends upon (i) the number of particles in suspension and (ii) their size.<sup>3</sup> Provided the latter is kept constant, *viz.*, by the production of suspension with uniform sized particles and comparison with an invariable standard of turbidity the intensity of the light scattered in a certain direction affords a measure of the amount of the suspended particles.

The method described here essentially consists in measuring the intensity of scattered light coming from an illuminated column of suspended substance\* with the light scattered from an invariable standard of turbidity, *viz.*, frosted glass discs, with the aid of a quantitatively variable light diminution. With a view to obtaining precision in measurements, and to correct for the differences in tint in the light reflected by the suspension and standard, the measurements are made within the range of any one of the appropriately selected colour filters blue (450–510  $\mu\mu$ ), green (520–560  $\mu\mu$ ), and red (620–680  $\mu\mu$ ).

From a calibration graph where the turbidity values are plotted against a series of known concentrations any observational reading may then be read off directly.

### *Apparatus*

The essential features of the apparatus may readily be seen from the schematic diagrams (Figs. 1 and 2).

The source of light L is a 30 watt 8 volt "Nitra" lamp supplying an intense light which is regular and easily adjustable. Two equal beams of light from the lamp enter through the two lenses A<sub>1</sub> and A<sub>2</sub>, the chambers C<sub>1</sub> and C<sub>2</sub> containing the turbid solution and the comparison standard respectively, in the form of a parallel pencil of rays square in section. The lights scattered by the suspended particles in the turbid liquid, and by the frosted

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\* The reaction producing the suspension should result in particles of uniform size and stability.

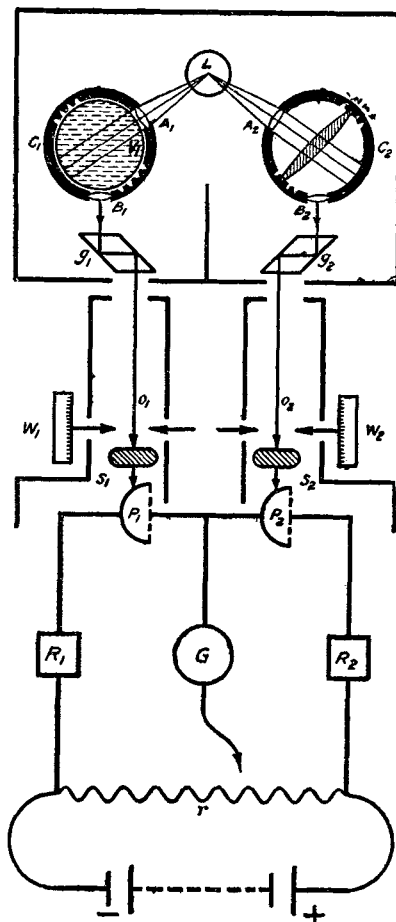


FIG. 1

Schematic diagram of Photoelectric Nephelometer  
The Vessel  $V_2$  is not shown in the figure

glass disc (standard) pass through the lenses  $B_1$  and  $B_2$  and the prisms  $I_1$  and  $I_2$  into the two measurably variable light apertures  $O_1$  and  $O_2$  (Fig. 2—to be described further) at an angle of  $45^\circ$  to the incidence. The light rays from the two apertures pass through  $S_1$  and  $S_2$ , the slots for the reception of the colour filters within the range of which a particular measurement has to be made. The lights from  $S_1$  and  $S_2$  then activate the photoelectric cells\*  $P_1$  and  $P_2$  respectively. The cells are continuously in circuit, the latter being of the bridge type employed by the authors in their previous instrument.<sup>2</sup>  $R_1$  and  $R_2$  are the protective resistances of 10,000 ohms each,  $r_1$  a variable

\* Cæsium cells (Becker & Co.).

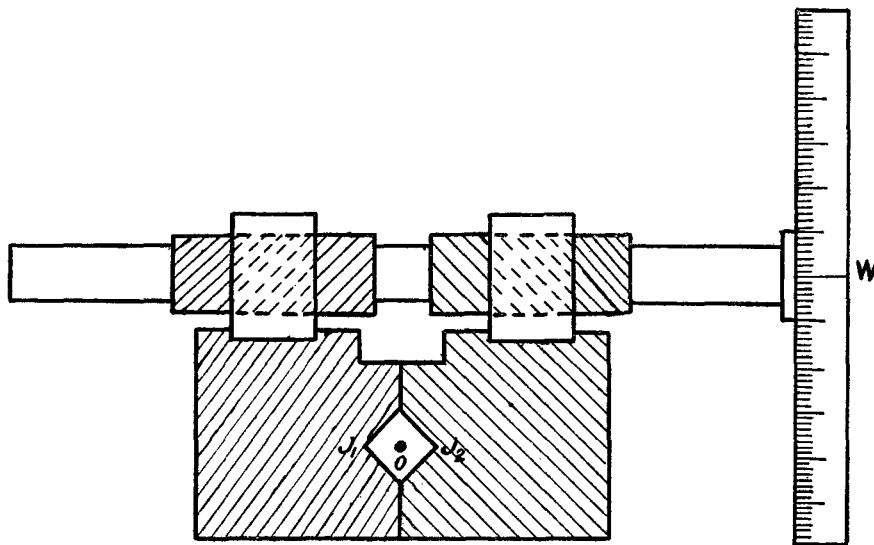


FIG. 2

FIG. 2. Variable light diaphragm of Photoelectric Nephelometer

resistance and G, the galvanometer with a sensitivity of 0.025 microamperes per mm. scale reading.

*Standard for comparison.*—The light used as a basis for comparison with the turbid medium passes through one of the four frosted glass discs of varying opacity (refer Fig. I, -1, 2, 3, 4). Each of these four discs is standardised by comparison with a body of translucent\* glass which is for all practical purposes invariable.

*Compensating mechanism.*—The light measuring device is a compensating mechanism of the nature of a variable light diaphragm (Fig. 2). Each of the apertures  $O_1$  and  $O_2$  (Figs. 1 and 2) can be measurably varied by means of the two V-shaped jaws  $j_1$  and  $j_2$  in such a way that the centre of the aperture always coincides with the optic axis. The ratio of the two amounts of light entering is proportional to the ratio of the areas of the two apertures. The scale readings† etched on the recorder W give a measure of the reflected

\* Supplied by Carl Zeiss Zena: Standardisation is effected by ascertaining the fraction of light which is dispersed in the opaque glass at an angle of  $45^\circ$  to the direction of illumination.

† If  $i$  = intensity of the reflected light (equals the area of the aperture),  $d$  = the variable diagonal between 0 and D of the square aperture and  $\alpha$  = angle between 0 and  $360^\circ$  through which W turns then,

(i)  $i = (d/\sqrt{2})^2 = (\alpha/360)^2$  for  $d$  from 0 to D and  $\alpha$  from  $0^\circ$  to  $360^\circ$  and,

(ii)  $\alpha = i \cdot 360^\circ = 0$  up to  $360^\circ$  for  $i$  from 0 to 1.

light as a percentage of the incident, 100 being the largest opening and 0 the smallest.

*Operation.*—The turbid fluid under examination is put into two convenient containers  $V_1$  and  $V_2$  (see below), and the latter inserted in chambers  $C_1$  and  $C_2$  and the light turned on. If the instrument is optically and electrically symmetrical, the galvanometer should show no deflection. But in practice this is not attained as slight differences in the arrangement are unavoidable and also on account of slight variation in the response of the photocells as no two photocells are identical. These slight variations are eliminated by the use of the common battery shunted by  $r$ . Hence using  $r$  as a fine adjustment the null-point is obtained. After this preliminary adjustment the light is switched out. The container with the turbid liquid is removed from chamber 2 ( $C_2$ , Fig. 1) and the light switched on again. Now on account of the difference in the intensities of the lights falling on the photocells the bridge is thrown out of balance and is indicated by the deflexion of the galvanometer (G). An appropriate frosted glass disc is then introduced\* into the respective slot of the chamber  $C_2$  such that the two intensities are approximately equal as indicated by the least deflexion of the galvanometer. The final compensation† is then effected by turning the appropriate recorder (left if turbid solution is brighter than standard and right if *vice versa*) till the null-point on the galvanometer is restored.

The measurement is repeated interchanging the positions of the turbid fluid and the frosted glass disc and the average of the two readings taken.

*Calculations.*—If  $o$  be the scale reading,  $t$  the opacity of the standard,  $d$  the value of the size of the vessel,  $S$  the correction factor for filter used (taking blue as standard) then opacity  $T$  of the turbid fluid is given by  $T = (o.d.t.) S$ .

Having obtained the value for opacity the concentration is read off from a calibration graph.

*Accuracy.*—A factor which greatly influences the accuracy of the determinations is the size of the vessel. Fig. 3 indicates the relationship between turbidity and concentration with different vessels. Needless to say that measurement with any particular vessel should be made in the region of linear proportionality, which nevertheless is of considerable extent.

The possibility of an imperfect return of the photocells to their original current output after having been exposed to the irregularities of light intensity associated with experimental procedures is likely to introduce errors.

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\* All four to be tried successively.

† The difference in the intensity of scattered light between the suspension and standard should be within a ratio 1 to 3. Otherwise, inaccuracies are encountered. In practice, this difference is not generally exceeded.

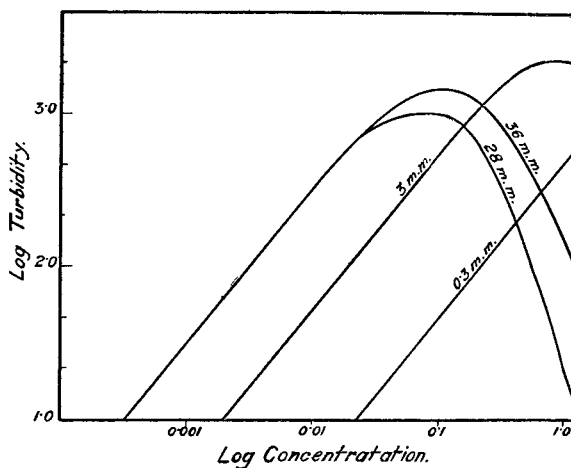


FIG. 3

Relationship between turbidity and concentration with different vessels

These can however be overcome by the selection of the proper filter, appropriate readjustment of the instrument and checking its readings at expedient intervals.

The reagent used should produce suspensions of equal dispersive power (equal sized particles) and also the suspensions produced should be fairly stable. This necessitates a preliminary testing of the possible precipitants and "protective colloids" for each substance, before successfully employing the apparatus.

The accuracy of the apparatus was tested by estimations of phosphorus following the method perfected by Kober and Egerer<sup>1</sup> for the production of a colourless, uniform and stable suspension. The method depends upon the production of a nephelometric cloud with the strychnine-molybdic acid reagent in HCl. The turbidity is proportional to the phosphorus content. The data are given in Table I.

A glance at the table shows that an accuracy of  $\pm 2.4$  per cent. is easily obtained in practice.

For the estimation of phosphorus in plant sap, 10 c.c. of the sap is digested with 20 c.c. of  $H_2SO_4$  (sp. gr. 1.84) and 8-9 gm. of  $K_2SO_4$  until a clear solution is obtained. Then the melt is cooled, dissolved in water and made up to 500 c.c. 25 c.c. of the solution are pipetted out, neutralized with  $NH_4OH$ , filtered through a dry, acid-washed filter paper, and the filtrate diluted to 200 c.c. 10 c.c. of this diluted solution are treated with the reagent, as given above and the suspension estimated after 4-6 minutes standing. On reference to a previously calibrated graph, the concentration corresponding to the observational reading is obtained.

TABLE I

*Determination of phosphorus with the Photoelectric Nephelometer**To 100 c.c. of potassium phosphate solution of the indicated concentration are added 30 c.c. of distilled water, 5 c.c. of 0.5 NHCl and 5 c.c. of strychnine-molybdic acid reagent*

Phosphorus present	Phosphorus found	Deviation	Error*
mg. per 100 c.c.	mg. per 100 c.c.		..
0.013	0.0132	+ 0.0002	1.6
0.016	0.0164	+ 0.0004	2.13
0.022	0.0215	- 0.0005	2.30
0.034	0.0334	+ 0.0004	1.7
0.041	0.0418	+ 0.0008	1.96
0.062	0.0612	- 0.0009	1.40
0.082	0.0811	- 0.0009	1.25
0.084	0.0820	- 0.002	2.4
0.090	0.0910	+ 0.001	1.2

\* Each an average of 10 readings.

*Advantages.*—Some of the special advantages of the new instrument are : the use of photocells eliminates the personal error in observations ; the activation of the two photocells by lights from a single source avoids the errors of instability due to intensity fluctuations in the light source and the measurement through a sensitive and objective method of photometry by physical means, in the form of a variable light diaphragm and within a well-defined region of the spectrum gives greater precision to the measurements.

*Applications*

The apparatus can be put to a variety of purposes :

(1) Determination of concentrations of substances following the procedure described for phosphorus in the preceding pages. For other organic and inorganic estimations that can be done with the apparatus the methods given by Yoe<sup>4</sup> are applicable with the necessary modifications.

(2) Chemical investigations of colloids, *viz.*, studying the variation in turbidity of a colloidal solution under varying conditions such as temperature, etc.

(3) Certain enzymic studies; the apparatus has been specially useful in these laboratories for the study of *Amylase* and *Lipase* by the method of Hans Kleinman.<sup>4</sup> The former depends upon a homogeneous and stable colloidal glycogen solution acted upon by *Amylase* and the undigested glycogen estimated; the latter depends in splitting a homogeneous and stable emulsion of triolein by *Lipase*, the course of digestion being followed by comparison of the digested and undigested triolein solutions. The results of these studies will form a separate communication.

### Summary

A new type of photoelectric nephelometer is described. The essential principle is the measurement of the intensity of scattered light coming from an illuminated column of turbid medium by a comparison with the light scattered from an invariable standard of turbidity (frosted glass) in a narrowly defined region of the spectrum with the aid of a quantitatively variable light diminution. Photoelectric cells are used to indicate equivalence of light.

The use of two photocells activated by light from a single source apart from eliminating the personal error in observations avoids the errors of instability due to intensity fluctuations of the light source. The measurement through a sensitive and objective method of photometry by physical means (in the form of a variable light diaphragm) and the use of light filters for measurements in narrowly defined regions of the spectrum give greater precision.

The accuracy of the apparatus is  $\pm 2.4$  per cent.

The several applications of the apparatus are described.

### REFERENCES

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