A STABILIZED PHOTOELECTRIC COLORIMETER WITH LIGHT FILTERS*

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Photoelectric colorimeters for biological work fall into two main classes. The first is the single photocell, direct reading photoelectric photometer, of which the Sheard and Sanford photelometer (6–8) may be taken as an example. The second is the double photocell, null point type, such as the comparison photometer of Goudsmit and Summerson (2). Quite apart from obvious advantages of simplicity of design and operation, the direct reading type is to be preferred, since it allows full use to be made of the advantages inherent in the application of color filters to the colorimetry of complex media. This type of instrument can, however, only be successful when it employs a source of light of absolutely constant intensity. This requirement introduces a technical difficulty which has been the cause of failure of most single cell colorimeters, and an attempt to evade the difficulty has led to the adoption of the double photocell principle. However, this does not by any means solve the problem of stability, since the resulting instrument exhibits erratic behavior as a result of unavoidable asymmetry of the response of the two cells to light of different wavelengths.

These, then, are the primary sources of error peculiar to the two types of photoelectric colorimeter, but superimposed on them is another fault shared equally by instruments of both classes, namely

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inconvenience of operation due to purely mechanical causes. The instrument to be described in this paper is a single photocell colorimeter in which extreme stability of light intensity has been achieved by a simple unconventional design. This design has not only eliminated errors due to light fluctuation, but has made possible a degree of simplicity and convenience of operation not hitherto achieved in any colorimeter, either visual or photoelectric. Moreover, the use of a truly constant source of illumination has made it possible to enlarge the field of application of light filters to cover most of the problems formerly requiring the use of the spectrophotometer, as well as other procedures for which not even the spectrophotometer is available. These new procedures will be described in a series of papers of which the present one deals with the design and operation of the apparatus, and indicates the nature and extent of its use in biological colorimetry.

General Considerations

The general principles of the single cell photoelectric colorimeter are briefly as follows: A beam of light (whose intensity can be varied at will, but which can be kept constant at any desired value) falls on a photocell which produces a deflection in a galvanometer to which it is connected. If an absorption cell containing a colored solution is placed between lamp and photocell, the percentage of light transmitted through the solution is proportional to the ratio of the final to the initial galvanometer deflection. The concentration of the colored substance in the sample can then be read from a chart showing the variation of light transmission with concentration. No assumption need be made as to the nature of the relation between light intensity and galvanometer deflection, nor between deflection and concentration. As long as the apparatus is constant in behavior, a colored solution which corresponds to a particular galvanometer reading at the time of calibration will give the same reading at any future time. Increased accuracy may be obtained by using color filters which transmit only that portion of the spectrum in which the solution has the correct degree of absorption (Kennedy (3), Exton (1), Sheard and Sanford (8), Koller (4), Millikan (5)). Indeed, the color filter technique can be refined to such an extent that it not only improves the accuracy of existing procedures, but renders entirely new ones possible.
FIG. 1. Diagrammatic cross-section showing electrical circuit. $A_1$, $A_2$, rectangular apertures 1 inch high by 7/8 inch wide; $B$, insulated binding post; $F$, glass color filter (Corning or Jena glass); $G$, galvanometer; $L$, Mazda No. 31 flash-light bulb; $P$, General Electric blocking layer photocell; $R$, 1.5 inch diameter aluminum reflector; $R_1$ to $R_6$, control resistances; $S$, 6 volt storage battery, dry battery, or voltage-regulating transformer; $S_{W_1}$, main lamp switch; $S_{W_2}$, low range lamp switch; $T$, 7 × 3/4 inch soft glass test-tube.
Description of Apparatus

In Fig. 1 is shown a schematic cross-section through the body of the colorimeter, together with a diagram of the simple electrical circuit.

A beam of light from the lamp \( L \) in the reflector \( R \) passes through the color filter \( F \), and then through the colored solution in the absorption tube \( T \), which is mounted in a suitable holder between the rectangular apertures \( A_1 \) and \( A_2 \) which define the cross-section of the light beam. The transmitted beam falls on the photocell \( P \), the current from which is indicated on the galvanometer \( G \). The lamp is energized by the power supply \( S \), and the intensity of the beam is controlled by the rheostats \( R_2 \) and \( R_3 \). The legend to Fig. 1 contains the essential facts about the more important components. One must, however, emphasize the fact that successful operation of the apparatus depends on rigid adherence to a definite set of optimum characteristics, not only in the electrical components but also in the mechanical assembly. Although such details cannot be given here, a few additional notes on the various components are required to make clear the more important features and advantages of the design.

Source of Light—This consists of a Mazda No. 31 flash-light bulb mounted in a hemispherical matte-surfaced aluminum reflector. The bulbs are individually selected for uniform physical and electrical characteristics, so as to be interchangeable without affecting the calibration of the instrument. The bulbs are run so far below their rated voltage that their useful life is equivalent to almost a year of hard service.

This simple arrangement has many important advantages. The power requirement (1 watt) and the current drain (200 milliamperes) are so low that the lamp can be energized by a 6 volt storage battery. This insures extreme stability over long periods of time. The small current in the lamp circuit also simplifies the problem of controlling the light intensity, since heating effects in rheostats are at a minimum. The light intensity can be varied over an extremely wide range (200-fold), thus allowing the use of filters of widely varying densities.

Power Supply—For best results and maximum stability a 6 volt

1 The author will be pleased to supply full constructional details and drawings to anyone who cares to write to ask for them.
lead storage battery is used. If this high degree of stability is not required, a standard 6 volt voltage-regulating transformer may be substituted, especially in localities where well controlled alternating current is available. Where portability is essential, satisfactory performance can be obtained with a 6 volt dry battery.

The light intensity can be varied smoothly over a wide range by the simple control circuit shown in Fig. 1. The only manual controls are the two switches and the rheostats $R_2$ and $R_3$ which provide coarse and fine adjustment respectively. The fixed resistor, $R_1$, limits the maximum voltage on the lamp to 5 volts, thus allowing an ample safety factor over the normal operating voltage of 6.2 volts. The switches $Sw_1$ and $Sw_2$ are of the low contact resistance, mercury-to-platinum type.

**Color Filters**—The glass color filters are mounted in brass filter holders (two filters to each holder) which can be moved up and down in a slot between the lamp and the absorption tube. Additional filters are kept on hand in extra interchangeable filter holders. Suitable filters for every problem can be prepared by using various combinations of different thicknesses of Corning and Jena glasses. These glasses are more stable than gelatin films, and are accurately reproducible by spectrophotometric standardization. The theoretical basis of the use of light filters in colorimetry, the mathematical theory of the photoelectric colorimeter with light filters (by analogy with spectrophotometer theory), and the technique of selecting filters for various problems will be fully discussed in later papers of this series. By careful selection it is possible to acquire a small set of filters which will cover a wide range of colorimetric procedures. In order to be able to select a combination of filters to isolate any desired region in the spectrum (down to bands only 40 $\mu$ wide), it is necessary to accumulate a large stock of various thicknesses of all the sharp cut-off filters available. As long as one such set of filters exists, it is unnecessary for the individual user of the colorimeter to make a collection of his own, since when once the correct filter has been chosen, it can be duplicated as often as necessary from the original data.

**Absorption Cell**—Instead of the conventional rectangular cemented glass cells, standard 7 $\times$ $\frac{3}{8}$ inch, round bottomed, soft glass test-tubes are used. The absorption tube fits into a bakelite sleeve which lines a brass tube, to the flattened sides of which the
other parts of the instrument are soldered. Interchangeable bakelite tubes with diaphragms of different sizes allow the use of samples of 6, 8, or 10 cc. instead of the usual 14 cc. Tubes of uniform dimensions are obtained from the makers, and final selection is made by filling all the tubes with the same colored solution, reading in the colorimeter, and discarding those which vary by more than 0.5 per cent from the mean. In this way one may easily obtain a set of 100 or more matched tubes which are inexpensive, convenient to handle, and easy to keep clean.

The use of standard test-tubes greatly simplifies the operation and construction of the colorimeter. Since many interchangeable tubes are available, one may carry out the entire preliminary chemical procedure (except in a few special cases) in the same tube in which the final colorimetric reading is to be made. As the act of making a reading does not in any way interfere with the solution under test, serial readings on large numbers of samples may be made as often as desired. This is particularly important in the case of volatile media. The ability to make rapid serial readings on numerous samples is invaluable in the study of the effect on color reactions of time, temperature, pH, and other variables.

Photoelectric Cell—The General Electric blocking layer photocell has been found most suitable. The cells must be selected to have approximately equal output under conditions existing in the colorimeter, but this is a convenience rather than a necessity, since each instrument is individually calibrated. By eliminating any possibility of heating effects, and by exposing the photocell to very low intensities of illumination (less than 1 foot candle), photoelectric fatigue and temperature effects have been rendered negligible. In this connection it should be pointed out that a great deal of the instability of certain photoelectric devices, commonly attributed to "photoelectric fatigue," is really due to heating effects in overloaded lamp circuits.

Galvanometer—The galvanometer should have a period of 3 seconds or less, a coil resistance of about 1000 ohms, and an external critical damping resistance of about 5000 ohms. A full scale deflection of 100 divisions should correspond to a current of about 2.5 microamperes. When many readings must be made at each sitting, a minimum of fatigue for the operator is assured by using an enclosed lamp and scale type of galvanometer, mounted on a
rigid shelf at the level of the seated observer's eye. The Rubicon type 3403 D.C. spot light galvanometer is most satisfactory. For a portable model a standard 15 microampere needle type meter (Weston, No. 440) is convenient. The photoelectric cell is connected directly to the galvanometer, for which it supplies a suitable external damping resistance. Since the calibration will be slightly influenced by the galvanometer coil resistance, interchangeable results will only be obtained when everyone uses the same type of galvanometer. This is of course not essential, as long as each apparatus is individually calibrated.
Assembly of the Apparatus—The main structural unit shown in Fig. 2 has no moving parts except the filter slide. A simple method of mounting the lamp and reflector makes it possible to change the lamp in a few seconds. The device may easily be duplicated in any workshop, and so long as the main unit is built rigidly to the proper specifications, the final assembly is entirely a matter of choice. All the components may conveniently be mounted on an aluminum panel which forms the cover of a wooden box; a door in the front of the box gives access to the interior when the lamp has to be changed (see Fig. 3).

Operation of the Apparatus

Before summarizing the very simple routine employed in using the colorimeter, it is necessary to call attention to one characteristic feature which is due to the use of a test-tube as the absorption
When a blank tube filled with a colorless solvent such as water is placed in the holder, it acts as a cylindrical lens which concentrates the light beam on the photocell. The galvanometer reading with a blank tube (referred to as the “blank setting”) is, therefore, greater than the corresponding reading with the holder empty (referred to as the “center setting”). The ratio between the two is about 1.5, and is determined by the geometry of the system, the wave-length of the light, and the refractive index of the contents of the tube. Since this ratio is constant for any one type of determination, it is immaterial whether the initial deflection is adjusted with the holder empty, or with a blank tube (containing solvent only) in place.

The operation of the colorimeter may now be summarized. The proper filter is selected, a blank tube containing pure solvent is inserted, and the rheostats are adjusted until an initial deflection of 100 divisions is obtained. The blank tube is now replaced by the sample tube, the new deflection is noted, and the corresponding value of the concentration is obtained from the proper calibration chart. After each reading the galvanometer returns to the original center setting, and the operator soon develops the habit of almost automatically checking its stability before inserting a new tube. This safeguard is valuable, although even this slight automatic readjustment of the setting should not be necessary in a series of twenty readings. The speed with which readings can be made is limited only by the time taken by the galvanometer to make the necessary excursion, so that ten readings per minute can be made with ease.

In calibrating the apparatus one makes up carefully and in triplicate a series of standards of different strengths to cover the desired range, and plots the best curve possible through the resulting points. No attempt should be made to read the galvanometer more closely than to the nearest half division, since this furnishes as much accuracy as can be expected from most colorimetric procedures. Since no color standards are required from day to day, the net result of this method of calibration is that the accuracy of each day’s results is maintained at a level corresponding to the greatest accuracy attainable under the most favorable conditions. In future papers dealing with various procedures which have already been investigated, full instructions will be given as
to the method of preparing a set of standards for calibration which will give an evenly spaced array of points.

Application of the Apparatus

A single pair of filters has been chosen with just the right degree of sensitivity to allow the apparatus to be used in place of the Duboscq colorimeter in all the standard colorimetric procedures, without the introduction of any modifications except the elimination of color standards.

When the most highly selective filters possible are used, the perfect stability of the readings allows accurate determination in extremely pale solutions. This has made possible a series of micromethods in which the useful range of various standard procedures has been extended far below the lower limits of visual colorimetry. The application of the apparatus to the accurate detection of the end-points of titrations is self-evident. The infra-red sensitivity of the photocell has encouraged the investigation of the "colorimetry" of nearly colorless solutions which have strong absorption in the infra-red but little or none in the visible region.

Studies of the behavior and velocity of color reactions are facilitated by the direct reading nature of the device, and by one's ability to make serial readings on large numbers of tubes in such a way that the act of making a reading does not in any way disturb the solution or necessitate the making of an adjustment to the apparatus before the next reading can be made. Rapid serial readings are invaluable in the quantitative determination of rapidly changing colors, since the point of maximum color development can be accurately determined.

By taking advantage of the wide range and reserve capacity of the illuminating system, highly selective filters can be used when it is necessary to eliminate the interfering effects of extraneous colors, or to determine independently both components of a mixture. It is in this field of heterochromatic colorimetry (for example, the analysis of mixtures of hemoglobin derivatives) that the instrument has found its widest application. Apart from its use for quantitative colorimetry, the apparatus may be used as a qualitative colorimeter for color matching and specification, by means of approximate trichromatic coordinates based on measure-
ments in three narrow spectral regions corresponding to the three primary colors.

Being essentially a photoelectric photometer, the apparatus may also be used as a nephelometer, especially in colored media in which the error due to absorption by the color itself can be eliminated by the use of suitable filters. For example, nephelometric measurements on red blood cell suspensions (made with a red filter to eliminate hemoglobin absorption) have been applied to the making of approximate rapid red cell counts, to the study of the kinetics of hemolysis, and to the determination of the fragility of red blood cells.

**Results**

The essential factors in the performance of the colorimeter on which data must be given are (1) precision (reproducibility of readings on duplicate samples), (2) accuracy (actual deviation from the true result), and (3) permanence of calibration (reproducibility of readings on the same sample from day to day). Data on all these points can best be given by reference to a single specific procedure. The determination of hemoglobin has been chosen because it involves no complex chemical manipulations, so that any errors which occur must be due entirely to defects in the instrument itself. Moreover, the Van Slyke oxygen capacity method provides a standard of known accuracy for purposes of comparison.

From the data presented in Table I, the following conclusions may be drawn with regard to the three factors listed above.

**Precision**—The figures in the second column of Table I indicate that the range of variation in samples set up in duplicate never exceeds 1 per cent. In terms of actual galvanometer readings this corresponds to one-half of 1 scale division. This may be taken as the maximum allowable variation, and is due almost entirely to practical limitations in the accuracy with which sets of matched tubes may be selected. None of this variation is due to random fluctuation in the light source, as this would never produce an alteration in the blank setting of as much as 0.05 division (in 100) during the time of making a reading.

**Accuracy**—The figures in the third column of Table I indicate that the probable error between the average of duplicate Van
Slyke and photoelectric determinations is of the order of ±2 per cent. This includes the operation of delivering 0.05 cc. of blood (from a calibrated pipette) into 20 cc. of diluting fluid. This degree of accuracy has been found in all of the other procedures in which there is a reliable method of establishing the true results.

**Permanence of Calibration**—The determinations whose results have been used in Table I have been chosen at random from our records over a period of about 2 years. There is no sign of any deterioration of performance, or of the development of systematic

![Table I](http://www.jbc.org/)

**Comparison of Hemoglobin Determinations by Van Slyke Oxygen Capacity Method and by Photoelectric Colorimeter**

The original calibration of the photoelectric colorimeter was made by means of Van Slyke determinations. Results are expressed as percentages of an arbitrary normal of 20.9 cc. of oxygen per 100 cc. of blood.

Duplicate determinations were made. The figures in parentheses represent the averages.

<table>
<thead>
<tr>
<th>Van Slyke</th>
<th>Photoelectric</th>
<th>Error per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>100.2</td>
<td>100.0</td>
<td>+0.6</td>
</tr>
<tr>
<td>94.3</td>
<td>93.5</td>
<td>-0.9</td>
</tr>
<tr>
<td>88.0</td>
<td>87.0</td>
<td>-1.4</td>
</tr>
<tr>
<td>112.5</td>
<td>110.6</td>
<td>-1.5</td>
</tr>
<tr>
<td>75.3</td>
<td>74.0</td>
<td>-0.6</td>
</tr>
<tr>
<td>78.8</td>
<td>80.0</td>
<td>+1.0</td>
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<tr>
<td>67.6</td>
<td>67.5</td>
<td>-1.5</td>
</tr>
<tr>
<td>108.0</td>
<td>106.0</td>
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<td>86.3</td>
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</tr>
<tr>
<td>70.2</td>
<td>71.5</td>
<td>+0.7</td>
</tr>
</tbody>
</table>

errors greater than the admitted limit of ±2 per cent. Possible causes of changes in calibration are deterioration of the lamp filament and small random changes in the spectral response of the photocell. Deterioration of the lamp is minimized by the low operating voltage; in addition, the lamp is checked against a standard lamp (never used for any other purpose) at intervals of about 3 months. Lamps are discarded as soon as deterioration is detected instead of waiting for them to burn out. The effect of small changes in photocell response can be rendered negligible by
adhering to certain definite principles in the choice of color filters. This will be dealt with fully in a future paper on the general theory of the use of filters in photoelectric colorimetry.

SUMMARY

A simple, easily constructed photoelectric colorimeter of the single photocell, direct reading type has been described. In this instrument, exceptional stability is secured by using a lamp of such low power requirement that it may be operated by a storage battery.

High illuminating efficiency obtained by the use of a reflector and smooth control of light intensity over a wide range permit the use of color filters of very high selectivity, thus greatly extending the scope of the apparatus.

In addition to the usual advantages inherent in the objective type of colorimeter, simplicity and convenience of operation have been improved by using standard test-tubes in place of conventional absorption cells.

Complete mechanical rigidity, absence of moving parts, and a large safety factor in all important components eliminate the usual causes of unsatisfactory performance.

The instrument has been used in the development of a number of new techniques involving analysis of mixtures of colored substances in solution. These will shortly be reported in detail.

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