PHOTOELECTRIC COLORIMETER

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In the photoelectric colorimetry of solutions, one of two factors may be the variable while the other is held constant: thickness of solution and intensity of transmitted light. The source of light is either considered constant or means are provided to compensate for its variations.

It is more difficult to use a device which depends upon the variation of solution thickness, for it is still necessary to read some electric meter to establish the relative transmission of light. Further, such arrangements do not advance visual colorimetry beyond replacing the eye with one or more photoelectric cells. The recently developed barrier-layer cell (1) has made possible an instrument which gives readings that bear a simple relationship to the concentration of solutions which conform to the fundamental Beer-Lambert law and does not require amplifying or other complex circuits which annoy the scientist as well as the technician who has little knowledge of electricity. Several variations of a simple instrument embodying these principles have been described, the most notable one being that of Müller (2). The instrument of Yoe and Crumpler (3), which also gives the transmission directly, is difficult to manipulate and requires large volumes and a delicate meter. All these instruments, however, specify optical systems which emit parallel or nearly parallel light. This specification is unnecessary and limits the instrument in two ways: first, the light-gathering lenses are limited in diameter to the width of the solution cell, resulting in low power with consequent limits upon (1) the thickness of the solution, (2) the handling of yellow solutions, and (3) ruggedness and cheapness of the meter; secondly, the volume of solution cannot be small.
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The photometering of yellow solutions is particularly difficult because their transmission in the blue end of the spectrum must be measured. The barrier-layer cell is relatively insensitive to blue light. This difficulty can be overcome by using a more concentrated beam of light.

Description and Operation of Apparatus

The instrument is shown diagrammatically in Fig. 1 and photographically in Fig. 2. B.L.C. represents the barrier-layer cell; S.C., solution cell; F, light filter; A, microammeter, 0 to 200 microamperes, 0 to 50 microamperes; $R_1$, coarse variable shunt; $R_2$, fine variable shunt; $R_s$, fixed shunt; $S_t$, tap switch; $S_t$, toggle switch.

The solution cell is an arrangement of two cells of equal thickness on a single slide. The thickness of solution which is most useful in biochemical determinations is about 1 cm. Additional cells of 0.5 cm. and 2 cm. increase the range of the instrument. The volume of solution is from less than 1 cc. to 5 cc.

One cell is filled with the solvent, the other with the solution under test. With the solvent in position, the meter is set by means of $R_1$ and $R_2$ to read a given current. The unknown is now slid into position and the meter read again. If the reading is low, the fixed shunt is thrown out by means of the toggle switch and the reading taken from the scale with the smaller range. As will be seen under calculations, it is advisable to have a meter whose scales are calibrated logarithmically, in which case the log current is read. The ratio of current for the unknown to that for solvent is the relative transmission. The lenses have short foci and large diameters. The source of light is a 32 candle power automobile lamp which draws about 4.2 amperes from a three cell storage battery. The storage battery should be maintained at full charge. A trickle charger will answer the purpose. The lenses are each set into screens which completely divide the case of the instrument, thus preventing conduction or convection of heat. Infra-red rays and possible change in spectral output of the light source are made inconsequential by the use of a filter.

The rise in temperature was determined for a combination of yellow solution and blue filter and a blue solution with a red filter. 2 cc. of a blood creatinine standard containing 0.06 mg. of creat-
inine in 30 cc. of final volume were placed in a solution cell of 1 cm. thickness. The bulb of a thermometer was placed in the

![Diagram](image)

**FIG. 1.** Schematic drawing of the apparatus and its optical system. B.L.C. represents the barrier-layer cell; S.C., solution cell; $F$, light filter; $A$, microammeter, 0 to 200 microamperes, 0 to 50 microamperes; $R_1$, coarse variable shunt; $R_2$, fine variable shunt; $R_a$, fixed shunt; $S_1$, tap switch; $S_2$, toggle switch.

solution, away from the path of light. The light, filtered through a Corning light shade, blue-green No. 428 filter, was passed
through the solution for 10 minutes, after which the solution was stirred with the thermometer and a reading taken. The temperature rose at the rate of 0.07° per minute. Under similar conditions the rise in temperature for a blue solution of Briggs' phosphorus, containing 0.83 mg. per 100 cc. together with a Corn-ing traffic shade, red No. 245 filter, was 0.26° per minute. The length of time required for a reading is about 1 minute.

In many methods, any appreciable change in temperature would result in very definite effects on the colorimetric reading, as, for instance, in a creatinine determination, where increase in temperature will cause a reaction by both creatine and sugar if these compounds are present in the solution.

**Method of Calculation**

A curve relating relative transmission and concentration is plotted on semilogarithmic paper with half a dozen solutions of known and differing concentrations. These points will lie on a straight line unless, because of ionization or other complex effects, the solution does not follow Beer's law. These effects are comparatively rare in colorimetric analysis. However, as the relative transmission in the region of absorption approaches 0, there is a deviation from Beer's law under the conditions of practical measurement. The range of analysis can then be extended by the use of a thinner solution. The use of a chart is not essential. Substituting into the line formula, \( y = mx + k \), the log of relative transmission for \( y \) and concentration for \( x \), we calculate the slope \( m \). \( m \) is determined for several values of concentration and the average taken. Unknown concentrations are now calculated from the formula \( c = \frac{(\log \text{ relative transmission} - k)}{m} \). If the meter is equipped with a logarithmic scale, the formula becomes simpler, \( c = \frac{(R_t/R_o) - k}{m} \). \( k \) represents the value of the blank and is usually 0. \( R_2 \), the reading for the solvent may be set at 100, the calculation then becoming \( c = 0.01R_t/m \) or \( c = R_t/n \), which holds for most analyses. Each combination of cell, filter, and method of analysis requires a different curve or \( m \). Once \( m \) is determined, its use or that of a curve is equivalent to that of as many duplicate standards as were used to find \( m \) or the curve. \( k \) should be rechecked for each new batch of reagents. \( k \) may be eliminated from consideration by using the reagent blank in place of solvent in the solvent cell.
Accuracy of Instrument

The accuracy of the instrument is a function of the slope of the curve. The slope may be sharpened by (1) increasing the thickness of solution and (2) selecting a filter which transmits primarily where the solution absorbs most.

Fig. 3 shows the effect of the color of light upon the acuteness of the slope of the curve for a series of blue solutions obtained in the determination of phosphorus by the method of Briggs (4). When white or blue light is used, the curve does not pass through the origin. This is due to the yellowish blank of the reagents. For ordinary work, a selection of three or four glass filters covering as many sections of the visual spectrum is all that is necessary. The precision of the instrument is such that duplicate curves determined several months apart agreed fully within the limits of error.

Some Analyses—The method of preparing a chart is illustrated in Fig. 3. Curves for the Benedict uric acid and sugar methods are very similar to that for Briggs' phosphorus. However, for the picric acid determinations, the curves are not straight lines throughout the range of analysis, but appear as two intersecting straight lines. Likewise, the curve for nitrogen consists of two such lines. Up to a concentration of 0.2 mg. of NH₃ in 100
cc. of final solution, there is one straight line; from 0.2 mg. to about 0.6 mg., the line continues straight but at another slope.

In Fig. 4, the transmission is taken relative to the reagent blanks rather than water. The data recorded here were taken with a single range, 0 to 200 microamperes, ammeter and a solution cell of 1 cm. thickness. In preparing the known solutions for plotting the curve, it is essential in this instance, and advisable generally, to add the same quantity of reagents to each solution and that the final volumes be the same, rather than to secure standards by the process of diluting the final solution. The Corning light shade, blue-green No. 428 filter was found satisfactory. It also serves well with the nitrogen determinations which produce a more definitely red color.

Table I shows the recovery of creatinine from a filtrate of human blood prepared in accordance with the Haden (8) modification of the Folin-Wu method. To varying amounts of filtrate were added varying quantities of creatinine standard, 1 cc. = 0.006 mg. The solutions were prepared on the basis of 20 cc. of filtrate or
20 cc. of a mixture including filtrate, standard, and water, to each of which 10 cc. of alkaline picrate are added. Table I is prepared on this basis, although the actual amounts taken were 0.35 of each ingredient.

By using a filter of narrower spectral range, such as the Corning No. 430, the accuracy may be increased.

**Table I**

*Recovery of Added Creatinine from a Blood Filtrate*

<table>
<thead>
<tr>
<th>Filtrate (cc.)</th>
<th>Standard added (cc.)</th>
<th>Transmission per cent</th>
<th>Total concentration as standard (cc.)</th>
<th>Filtrate concentration as standard (cc.)</th>
<th>Standard recovered (cc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0</td>
<td>62.3</td>
<td>6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10*</td>
<td>0</td>
<td>64.5</td>
<td>3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.3</td>
<td>5.7</td>
<td>59.5</td>
<td>10.0</td>
<td>4.5</td>
<td>5.5</td>
</tr>
<tr>
<td>17.2</td>
<td>2.8</td>
<td>60.7</td>
<td>8.3</td>
<td>5.4</td>
<td>2.9</td>
</tr>
<tr>
<td>11.4</td>
<td>8.6</td>
<td>58.0</td>
<td>12.3</td>
<td>3.6</td>
<td>8.7</td>
</tr>
</tbody>
</table>

* Plus 10 cc. of water.

**Table II**

*Agreement between Duplicates of Yellow Solutions*

<table>
<thead>
<tr>
<th>Concentration, NH₃ per 100 cc.</th>
<th>Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duplicate 1</td>
</tr>
<tr>
<td>mg.</td>
<td>per cent</td>
</tr>
<tr>
<td>0.02</td>
<td>94</td>
</tr>
<tr>
<td>0.06</td>
<td>90.5</td>
</tr>
<tr>
<td>0.10</td>
<td>86</td>
</tr>
<tr>
<td>0.16</td>
<td>81</td>
</tr>
<tr>
<td>0.20</td>
<td>77.3</td>
</tr>
<tr>
<td>0.40</td>
<td>66.5</td>
</tr>
<tr>
<td>0.60</td>
<td>58</td>
</tr>
</tbody>
</table>

The blood creatinine standards gave a curve of sharper slope by a very slight modification; instead of diluting the standard to 20 cc. as called for in the method, dilute to 4 cc. and add the usual 10 cc. of alkaline picrate reagent. This is not objectionable in view of the small quantity of solution we require. In applying this modification to blood filtrate, 4 cc. of the usual filtrate may be taken.
Nitrogen was determined by the method of Folin and Bell (9). Mr. Myles Braver, of this laboratory, made the determinations presented in Table II in the course of his regular work. They are presented to show the extent of agreement between duplicates. The duplicate readings were taken on different days. A 1 cm. solution cell and a Corning filter No. 428 were used.

The time required for taking the reading, rinsing the cell, and refilling it for the next determination is about 1 minute. A convenient device for emptying the cell is a rubber bulb with a glass tube which is drawn to a fine bore.

**SUMMARY**

A photoelectric colorimeter is presented which (1) has wide range, (2) is accurate at high and low concentrations, (3) requires less than 5 cc. of solution, (4) is easily and rapidly manipulated, (5) gives satisfactory results with a rugged and inexpensive meter even when used with yellow solutions, and (6) its readings bear a simple relationship to the concentration of solutions which conform to Beer's law. A fixed shunt extends the scale of the meter.

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