# COLORIMETRY *for* CHEMISTS

on loan from



**M. G. MELLON, PH.D., SC.D.**
Professor of Analytical Chemistry
Purdue University

Published by
THE G. FREDERICK SMITH CHEMICAL CO.
867 McKinley Ave.
COLUMBUS, OHIO

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# PREFACE

Analytical data may be secured in a surprising number of ways in a well-equipped chemical laboratory. For a specific sample the property measured and the measuring instrument employed depend upon the situation, such as the physical state of the sample, the amount available, the time required, and other factors. Instruments vary from simple equipment, such as hydrometers, to the new and complicated mass spectrometer. In any case, one measures either a specific property of the desired constituent, such as mass, or a systemic property of the material containing the constituent, such as color.

In chemical usage the term colorimetry includes both measurements of color as color, and of color (strictly light-absorptive capacity) as related to concentration of some desired constituent. With the addition of a variety of equipment employing photocells, this division of analytical chemistry now has probably an unsurpassed number of instruments. It is beyond the scope of this brief book to present for all such equipment a detailed description of the mechanical, optical, electrical, and operational features, including the advantages and disadvantages of the various modifications and the applications of each.

Briefly, the objective is to present an outline of colorimetry, as currently applied in chemistry, in order to provide a perspective of the problem involved and of the means of meeting it. The past two decades have brought hundreds of publications on such analytical methods and apparatus. References are given to representative sources for more detailed information.

Unfortunately, the literature of colorimetry abounds in confusing usage, particularly with respect to terms, symbols, and the manner of presenting data. In general, an attempt has been made to conform with the recommendations of the report of the Spectrophotometry Committee of the Optical Society of America, and of the report, as far as it has appeared, of the new Colorimetry Committee of the same organization.

M. G. MELLON.

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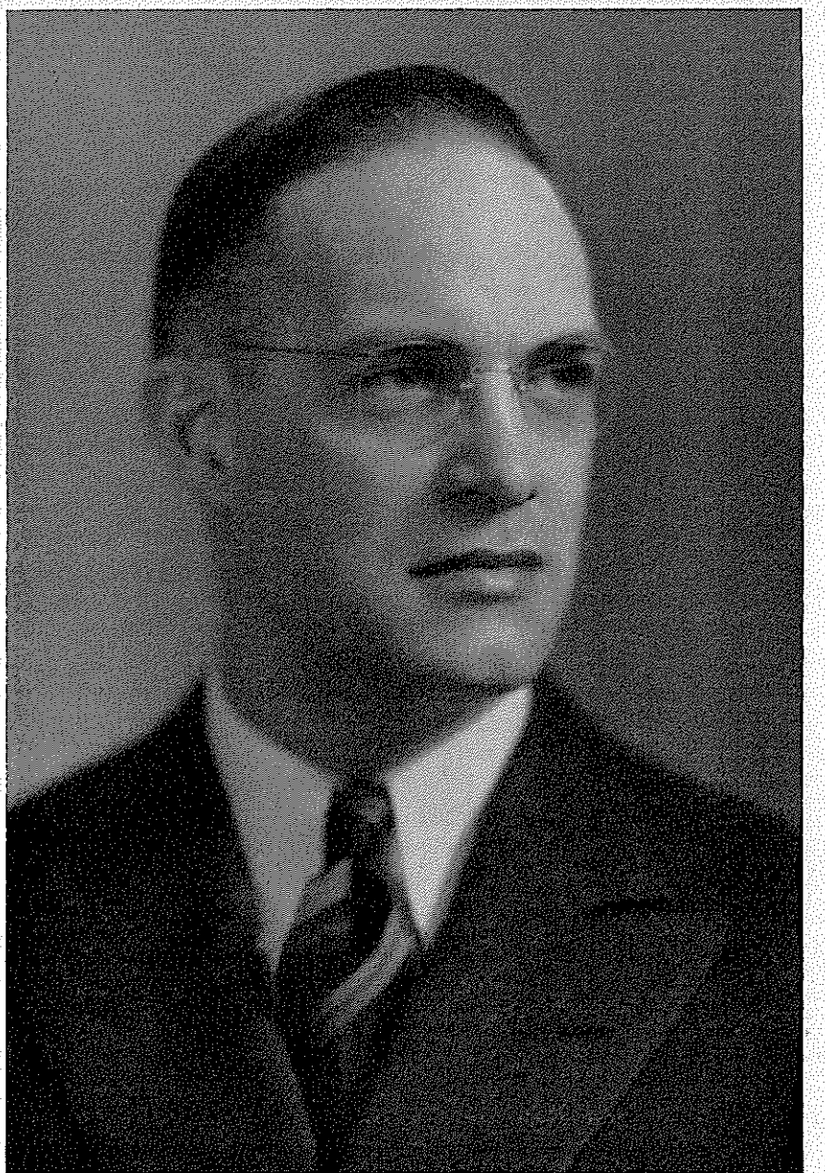

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Professor of Optics and Photography
Massachusetts Institute of Technology

Inventor of the recording photoelectric spectrophotometer
manufactured by the General Electric Company.

# Colorimetry for Chemists

## CHAPTER 1

## INTRODUCTION

### DEFINITIONS AND PERSPECTIVE

The identification and/or determination of constituents by the methods of analytical chemistry depend upon the recognition and measurement of certain characteristics known as physical properties. Thus, solubility, volatility, odor, and similar attributes serve for qualitative characterization; and mass, volume, density, and various other properties serve for quantitative measurement.

Color is usually considered as one of these properties of materials. Analysts are familiar with its wide use as a means of detecting constituents. In quantitative analysis recent years have brought a wide and rapidly increasing use of colorimetric methods of measurement. Thus, it has both qualitative and quantitative uses. Although these are perhaps sufficiently extensive in themselves to make this one of the most useful of analytical properties, there is still another application which may be designated as the measurement of color as color.

Among the properties widely used for the analytical evaluation of materials, color is unique in an aspect important from the standpoint of the usage of terms. While every material possesses the specific property of mass, no material is actually colored as such, in strict usage of terms. Yet we are surrounded with objects and phenomena to which we attribute color. Because of the desirability of clear analytical application of terms, it is necessary to recall the present concept of color *(34a)** and to define certain terms *(34b,c)*.*

Color is related, in a way, to some source of radiant energy (the illuminant), to the object to which we ascribe the color, and to the

* NOTE: These papers are parts of a comprehensive report of the new Committee on Colorimetry of the Optical Society of America, which ultimately are to be published under the title, "The Science of Color." As tentatively announced, the following sections are to be included: I. From the art of coloring to the science of color; II. The concept of color; III. The human eye; IV. Psychology of color; V. Physical concepts: radiant energy and its measurement; VI. The psychophysics of color; VII. Quantitative data and methods of colorimetry; VIII. Colorimeters and color standards; IX. Summary (glossary and index). Parts II, V, VI, VII, and VIII have been published as separate papers, listed in Ref. 34 as *(a)*, *(b)*, *(c)*, *(d)*, *(e)*, respectively. It should be noted that the whole report is a joint product of the action of representatives of the various groups concerned with color and its applications, such as physicists, chemists, psychologists, and artists.

eye of the individual who observes the color. The radiator, or light source, emits energy which travels through space in the form of electromagnetic waves of various lengths. The "colored" object reflects or transmits selectively this radiant energy incident upon it. In the eye of the observer the radiant energy from the object is received on specific components of the retina, the rods and cones, as a result of which the normal individual experiences the sensation of color. In reality, then, the color is in the observer, rather than in the "colored" object. The property of the object with which we seem to be concerned is its light-absorptive capacity. Because we do so generally attribute color to such objects, this implication will be followed here. If a photocell, rather than the human eye, is the instrumental receptor of the radiant energy from the object, obviously the measuring instrument will not indicate any sensation of color.

According to approved definitions, light, the stimulus of colors, "is the aspect of radiant energy of which a human observer is aware through the visual sensations which arise from the stimulation of the retina of the eye" *(212)*. Since it is thus limited to the wavelengths of radiant energy characterized by visibility, " the expressions ultraviolet light and infrared light are misnomers" *(68)*. It may be noted that this psychophysical definition does not identify light with either radiant energy or visual sensation.

As recently defined *(34d)*, "color consists of the characteristics of light other than spatial and temporal inhomogeneities."

The sensation of color is evoked by radiant energy reaching the retina if the spectral distribution throughout the visible region is unequal. If it were equal, the light would be white, or like sunlight. This unequal distribution may be characteristic of the source itself, or it may be the result of selective absorption by the system which appears colored. Examples of the first type are flame spectra, composed of one or more monochromatic wavelengths. The analyst's chief colorimetric interest in these colors is the possibility of distinguishing visually between certain chemical elements. Examples of the second type are the great number of systems which show selective absorption for light and exhibit color as a result of reflection or transmission of the unabsorbed incident radiant energy. Thus, if a ribbon illuminated with daylight appears green, it is because the proportion of light reflected from the middle of the spectrum is much greater than that at the ends. The reverse of this situation, except that the light is transmitted, accounts for the purple of an aqueous solution of a permanganate.



It has already been noted that color involves three entities—the radiator, the object, and the observer. This number three is of further significance in colorimetrics. There are three factors of importance in connection with the radiant energy emitted by the radiator—the spectral quality, the angular distribution, and the intensity. Then there are three characteristics of this light in terms of which color may be specified—dominant wavelength, purity, and luminance. These characteristics correspond, in a general way, to hue, saturation, and brightness, the three attributes in terms of which color sensation is described. Finally, in the calculation of the numerical characteristics of light, use is made of three tristimulus values (red, green, and violet).

Although the principal consideration of these subjects will be found under the discussion of color analysis, occasional use of some terms before that point warrants a brief statement here. The usage follows largely that in the recent reports of the Committee on Colorimetry of the Optical Society of America.

The *dominant wavelength* of a sample is the wavelength of spectrally homogeneous radiant energy which would have to be mixed with an appropriate amount of achromatic, or "white," light in order to match the chromaticity of the sample. The *purity* is an expression of the proportion of the spectrally pure component in the mixture matching the chromaticity of the sample. *Chromaticity* refers to the characteristics specified by dominant wavelength and purity. *Luminance* is not easily defined to cover all its aspects. It will suffice here to consider it, in relation to reflectance and transmittance, as the ratio of the light leaving the object to that incident upon it.

Next come terms relating to the sensation experienced by the observer. *Brightness* is that attribute of any color which permits it to be classified as equivalent to some member of the series of achromatic colors (grays, ranging from black to white); *hue* is that attribute of certain colors which permits them to be classed as reddish, yellowish, greenish, bluish, or purplish; chromatic colors exhibit hue, but achromatic colors do not; and *saturation* is that attribute of a chromatic color which determines its degree of difference from the achromatic color of the same brightness.

The current literature of analytical chemistry and optics shows that the term colorimetry may have quite different meanings for different individuals. To the physicist it implies measurement of color in the sense of determining the magnitude of the three values, dominant wavelength, purity, and luminance, or of the trichromatic

coefficients, red, green, and violet *(83)*. His interest is color analysis. To most chemists colorimetry has long implied measurement of the amount of a constituent by comparison of the colored system containing the unknown with a similar system containing a known amount of the desired constituent, or with a system visually equivalent to the latter. In this sense, obviously chemists' colorimeters are only comparators. In recent years absorptometers are often included along with the chemists' comparators. The more general viewpoint presented here is intended to correlate, at least for analysts, the interests of both physicists and chemists in the problem of measuring and specifying colored systems.

**Color in Chemical Analysis.** An outline of the general significance of color in analytical chemistry was proposed in another publication *(146)*. Consideration of these possibilities shows that the quantitative measurement of color, like a number of other physical properties, comprises two principal types of uses: (a) observation or measurement of the color as such, including determinations of colorimetric specifications, such as the combination of stimuli required to match a given color; and (b) the detection and/or estimation of some constituent in a sample. Just as a metallurgical laboratory may determine hardness and carbon content of a steel, a testing laboratory may measure dominant wavelength and copper content of a sample.

Since chemical analysis generally has wider interest than colorimetric specification in the analytical laboratory, the subject has been treated accordingly here. It has seemed best to classify the material on the basis of the method of measurement used. Then both analysis and colorimetric specification may be taken up in a given section.

**The Domain of Colorimetry.** In its broadest applications, colorimetry includes all procedures which have as their objective the evaluation of materials by means of some property related to color. The measurements divide themselves into those dealing with the determination of color as color, and with the determination of the presence and/or the amount of some desired constituent. Systems possessing the characteristic we designate as color comprise most of the materials surrounding us, including gases, liquids, and solids.

Measurement of the colors of gases is not often made, although it is possible, for example, to estimate nitrogen dioxide roughly in this manner. Practically all measurements on opaque solids are by reflection, usually with the object of establishing an objective specification, either in terms of spectral reflectance curves or of some specified stimuli. Analysts are most concerned with colorimetry of



liquids, and especially solutions. In this case the transmittance or absorptance of light is now measured most often.

Although liquid solutions may be measured to determine one or more colorimetric specifications, generally the object is to estimate some desired constituent, which may be considered as the solute. If such a constituent is to be determined in this way, either it must itself possess suitable colorimetric characteristics such as those of the permanganate ion, or, as much more frequently happens, it must be capable of reacting with some reagent to give a substance having suitable colorimetric characteristics, such as those possessed by an aqueous solution of chlorine after reaction with o-tolidine. Such possibilities are outlined later.

**Applications.** Presumably every colored object is susceptible of colorimetric specification, provided the color is stable long enough for measurement. Paints and dyes are two familiar examples, but the list might be extended almost indefinitely to include a great variety of articles of commerce.

The extent to which quantitative determinations of constituents have been based upon colorimetric properties of solutions is shown in the treatises of Yoe *(244)*, Sandell *(182)*, and Snell and Snell *(196)*. The last work, comprising two large volumes, includes over 900 methods, in which approximately 700 different reagents are used for nearly 400 elements, radicals, and compounds. Many papers are appearing each year to increase our knowledge of this subject, which at present is one of the most active in quantitative analysis.

These colorimetric methods can be applied to a variety of systems in fields ranging from metallurgy to medicine, the clinical laboratory being a notable example. Such procedures are applied extensively to industrial products from foods to steel. In water analysis, for example, they have long been official for a number of constituents.

**Merits of Methods.** A comprehensive appraisal of the merits of all common methods of measuring color, whether for colorimetric specification or chemical analysis, has not been attempted here. As with any other kind of method, detailed consideration should deal with items such as the following: cost of the equipment and its use, service facilities required, skill required in the operator, time required for a measurement, amount of sample needed, effect of the measurement on the sample, preliminary treatment of sample needed, sources of error, and the reliability of the results. Only a few general statements are included. Some points are mentioned more specifically in connection with particular kinds of methods.

The general merit of numerical colorimetric specification may be summarized by stating that they provide a definite basis for recording and transmitting information concerning colors. The reliability of the results is likely to be directly related to the objectivity of the method of measurement used.

For chemical analysis the range of sensitivity of different methods is quite variable. Some are sensitive to one part per billion (an exception, of course), while others are applicable to 10 to 20 parts per million (also an exception). Many methods apply over a range from about 0.5 to 10 p.p.m. The range of application is determined by the intensity of the color of the system to be measured, including our control over it, and by the sensitivity of the means of measurement used. With very low and with very high concentrations of desired constituent small differences in amounts can not be determined reliably. Such methods usually are inapplicable, without dilution, to quantities greater than one per cent of the total sample, although Mehlig, using a spectrophotometer, worked with much larger amounts *(135, 136)*.

The absolute accuracy differs with different methods of measurement, and in some cases with the nature of the color of the system measured. With visual comparators it is usually within 5 per cent of the amount present. Well designed photoelectric photometers, on account of their increased sensitivity, may give a somewhat lower error. Some of them will reproduce transmittance measurements to 0.1 to 0.2 per cent.

The equipment used in analytical determinations may be very simple and cheap, especially for crude colorimetric comparisons. Even good Nessler tubes or optical comparators are relatively inexpensive. Such equipment does not require much knowledge nor experience on the part of the operator and few quantitative determinations require less ability. Determinators and technicians are at their best here. In the other direction hardly any analytical apparatus is more expensive than certain photoelectric spectrophotometers, and they require a highly skilled operator to obtain reliable data, especially when adjustments of the instrument have to be made.

Colorimetric and titrimetric methods are comparable in that often the measurement may be made without performing any previous separation of the desired constituent. Thus, one may determine residual chlorine in water by adding the reagent o-tolidine without regard to most other constituents likely to be present. In all cases, of course, one must know that the color to be measured is to be attributed



entirely to the desired constituent, unless provision can be made for the cases in which it is not true.

**Methods of Measurement.** Since the word colorimeter is not used in the same sense in physics and chemistry, the author suggests that it be applied in each field in a general sense for any instrument that measures a property which is a function of one or more of the attributes of color. Then instruments having a special application may be designated by more specific terms, as indicated later. Using the term in the sense proposed, colorimeters include instruments applicable to the visible region of the spectrum but not those for the ultraviolet or infrared regions.

Many chemists are probably not fully aware of the number and the variety of the devices which have been proposed for measuring different characteristics of colored systems. The last two decades have brought notable advances in the introduction and improvement of such instruments. This presentation is an outline of the most important kinds of apparatus now being used for colorimetric measurements in analytical work. The classification follows that proposed earlier *(140)*. Specific commercial instruments are mentioned as illustrative of a class rather than as necessarily the best of a given type.

So many different measuring instruments and methods of measurement have been suggested that the space available permits only a sampling of the material. This statement of the present status of the subject includes mention of representative examples of instruments and methods, together with their applications and merits. Some topics have had to be treated so superficially that the references cited must be consulted for details. For details of instrumental operation, and often also of construction, the reader should consult the respective manufacturer's technical bulletins or the original articles, if any have been published.

**Sources of Error.** Anyone experienced in physical measurements would predict from the wide variety of instruments discussed that there would be many possibilities of error in using such equipment. Actually these possibilities are so numerous that adequate emphasis can not be given all of them in the space available.

Some errors are more or less peculiar to a particular instrument, or at least to a certain type of instrument. For example, one need be concerned with characteristics of photocells only when working with a photoelectric instrument. Again, only visual instruments involve the defects of the human eye as a receptor for radiant energy. When given instruments are discussed, the more important of these

sources of error are mentioned. Technical bulletins of the manufacturers may be expected to be more comprehensive in this respect. Even then, actual experience with the more complicated instruments is probably the best insurance against error.

## THE CHEMISTRY IN COLORIMETRY

Strictly speaking, colorimetry is measurement, and therefore involves no chemistry. Actually, in the determination of a desired constituent, one seldom finds the system originally in shape for measurement. The determination of aluminum in a magnesium-base alloy is an example. First the sample must be dissolved. Then the aluminum ion, since its solution is colorless, must be converted into a colored system having suitable colorimetric properties. In case any other constituent interferes, one must compensate its effect, remove the constituent, or prevent its functioning through some appropriate chemical reaction. These transformations constitute the chemical portion of the analytical procedure *(143)*.

Unless the sample needs no preliminary treatment, one must first prepare it for measurement. The present objective is to consider briefly some of the chemical problems involved. The most important of these are the characteristics of colored solutions, the selection of color-forming reactions, and the provision for interferences.

**Desirable Colorimetric Properties.** Occasionally the system to be examined is already in a form suitable for measurement without making any chemical transformations; but since this is so rarely the case, we shall assume that such treatment is necessary. Also we shall assume that the measurement is to be made on a liquid solution containing the desired constituent, or another constituent which is chemically equivalent.

Before considering the possibilities of preparing such colored systems, we may note the general requirements which they should meet to make them most satisfactory for use. Relatively few solutions, of course, approach the ideal of meeting all these requirements. Knowing the requirements, the wise analyst selects from the systems available the one best adapted to his problem, and then he uses it with the knowledge of what the deficiencies are.

In general, any solution destined for colorimetric measurement should possess at least the following properties:

1. Be intensely colored—so that small amounts of the unknown may be determined. In many cases flexibility may be achieved through preliminary dilution or concentration of the desired constituent. Also different color-forming reagents, if used, may be



found for application to different ranges of concentration. Some solutions are subject to color intensification by altering the nature of the solvent system, as in the use of acetone when determining iron as the thiocyanate.

2. Be stable—so that the determination need not be completed rapidly before fading would vitiate the results, and so that similarly prepared standards for visual matching will be reasonably permanent. Instability is the result of several kinds of action, such as air oxidation and photochemical irradiation. Control of the operative factor often enables one to prepare a measurable system.

3. Be little affected by pH change—so that close control of this factor is unnecessary. If pH changes do affect the color, it is desirable to be able to secure a measurable system by adding an approximate amount of some common acid or base, or a compound such as ammonium acetate or borax. With very sensitive systems, one may have to resort to close buffering, usually checked best by means of a glass electrode.

4. Have a color which permits a spectral region between 475 and 625 mμ to be isolated for measurement, if visual means are used—so that advantage may be taken of the maximum position of the relative luminosity curve. With photoelectric instruments, the region of maximum sensitivity will depend upon the photocell.

5. Have the system possess a small temperature coefficient (and also have any necessary color formation proceed at room temperature)—so that the procedure may be as simple as possible.

6. Have the colorimetric characteristics reasonably close to those of the "matching" permanent standards, if such are used—so that comparisons will be valid under any lighting conditions.

7. Conform to Beer's law—so that any type of method of measurement may be used.

In addition to these general requirements, systems resulting from reactions with color-forming reagents should meet, as far as possible, the following requirements:

1. Develop the color quickly—so that one need not wait on a reaction taking much time for its completion.

2. Form the color with a reagent which does not itself show selective light absorption—so that excess reagent will not complicate the method. Use of a colored reagent gives a system whose total color depends upon the excess reagent added.

3. Have a color reaction which is free of interference by substances other than the unknown—so that the color formed will depend

only upon the desired constituent present. Ideally, we want a specific reaction. Practically, one of high selectivity is the best we achieve.

4. Have the intensity of the color produced independent of the amount of the color-forming reagent, provided an excess is used—so that the amount of the desired constituent may be assumed to be proportional to the intensity of color (providing Beer's law applies). If the reaction is not stoichiometric, use of a large excess of reagent may yield reasonably satisfactory results.

5. Have the nature of the color-forming reaction known—so that reproducible conditions may be maintained if the process is susceptible to the effects of variable factors.

6. Use the same solvent for the unknown and for any color-forming reagent—so that excess reagent will not itself precipitate nor cause precipitation of high concentrations of diverse ions.

7. Require no special treatment for the colored solution, such as extraction of the colored compound with an organic solvent—so that the procedure may be as simple as possible.

8. Be independent of the order of mixing reagents—so that the routine of operations is not critical.

**The Colored System.** Colorimetric measurements are applicable, of course, to gases, liquids, and solids. In analyzing gases one may find a naturally colored constituent, such as nitrogen dioxide, but such instances are rare. Somewhat more frequently liquid samples may contain a desired constituent in a suitably colored form. In the great majority of cases, however, the sample is a solid. Usually it must be dissolved, although a volatilization reaction may effect the separation of a constituent which can then be converted into a colored system. Unless volatilization is applicable, dissolution is the alternative. The latter operation is so well known in analytical work that it need not be considered here. Measurement may be practicable on the solution as such, particularly in the case of organic compounds; but much more frequently a measurable color must be developed by means of some chemical reaction.

Very useful compilations of what has been done in this direction are contained in the treatises by Yoe *(244)*, and by Snell and Snell *(196)*. The later books by Feigl *(51)*, Mellan *(138)*, von Stein *(223)*, and Yoe and Sarver *(247)* summarize the general use of compounds as analytical reagents, including the formation of soluble, colored systems. The papers by Diehl *(38)*, Feigl *(52)*, Haendler and Geyer *(80)*, and Sarver *(184)* help to systematize our knowledge of the way in which the organic compounds react, particularly in the formation of chelate ring structures. Sandell's book *(182)* covers the use



of selected reagents for the determination of the metals. The common metals are covered also in Allport's book *(2a)*, but the emphasis is on organic materials.

It seems to be desirable to have a general outline of the kinds of chemical reactions which have been found useful in developing colors suitable for colorimetric measurement. With it one should be helped, not only in keeping in mind what has worked, but also in selecting possible new reagents. In extrapolating past experience, however, we must be careful not to leave the laboratory long to theorize on what we think should happen with an untried reagent. Thus, certain cyclic nitrogen compounds, such as 2,2′-bipyridyl, are excellent reagents for ferrous iron; but some closely related derivatives, containing the same chelating group, do not work. Incidentally, we are interested both in systems possessing color, and in those which will fluoresce characteristically under certain conditions.

The accompanying outline is the result of an effort to classify important means of securing colored solutions. It seemed convenient to subdivide the desired constituents into inorganic and organic groups, and then, as far as possible, to consider the reactions in terms of elements, compounds, and ions.

It will be noted that no distinction is made between reactions yielding solutions and those forming precipitates. In fact, precipitation may be necessary to effect the desired color formation. The material is then brought into solution, by means of an appropriate solvent, to render the system measurable. Thus, potassium is precipitated as the dipicrylaminate, filtered, and dissolved in acetone. If simple dissolution of the precipitate does not yield a satisfactory colored system, it may be possible to enhance or improve the color by additional reactions. For example, the oxine from precipitated and dissolved oxinates may be coupled with a diazo compound or oxidized by certain heteropoly acids to give colors.

### I. Inorganic Constituents

A. Colored as such

1. Elements
   e.g.—$2\ Br^- + Cl_2 \rightarrow Br_2$
2. Compounds
   e.g.—$NO_2$ in gases
3. Ions
   e.g.—$MnO_4^-$, $Cr^{+++}$, $Cr_2O_7^{--}$

B. Colorless (Color must be developed)

1. Elements
   e.g.—$Cl_2$ in water + o-tolidine → color

2. Compounds
e.g.—$H_2O_2 + MoO_4^{--} \rightarrow$ color

3. Ions
a. Inorganic reagents
a.′ Oxidation
e.g.—$Mn^{++} + IO_4^- \rightarrow MnO_4^-$
b.′ Reduction
a.″ Directly
e.g.—2 $Au^{+++} + SnCl_4^{--} \rightarrow$ colloidal gold
$H_3[P(Mo_3O_{10})_4] + SnCl_4^{--} \rightarrow$ Mo-blue
b.″ Indirectly
e.g.—$Ca^{++} \rightarrow CaC_2O_4 \rightarrow Ca_3(PO_4)_2 \rightarrow$
$H_3[P(MO_3O_{10})_4] \rightarrow$ Mo-blue
c.′ Complexation
a.″ Cations
e.g.—$Cu^{++} + 4\ NH_3 \rightarrow Cu(NH_3)_4^{++}$
b.″ Anions
e.g.—$Co^{++} + 4\ NCS^- \rightarrow Co(NCS)_4^{--}$
d.′ Decolorization
e.g.—$Fe(NCS)_6^{---} + 6\ F^- \rightarrow FeF_6^{---}$
b. Organic reagents
a.′ Chromophoric transformation
e.g.—$H^+$ + pH indicator → color change
b.′ Salt formation
e.g.—$HNO_3$ + phenol-2,4-disulfonic acid + KOH → color
c.′ Coupling
e.g.—$HNO_2$ + sulfanilic acid + α-naphthylamine → color
d.′ Adsorption
e.g.—$O_2$ + 2 $I^-$ + starch → color
$Al^{+++}$ + quinalizarin → lake
e.′ Oxidation
e.g.—$Cl_2$ + 3,3′-dimethylbenzidine → color
f.′ Reduction
e.g.—$H_3[P(Mo_3O_{10})_4]$ + hydroquinone → Mo-blue
g.′ Fluorescing formation
e.g.—$Al^{+++}$ + morin → fluorescing system
h.′ Chelation
a.″ Two coordinate bonds
e.g.—$Fe^{++}$ + 2,2′-bipyridyl → color
b.″ One coordinate and one electrovalent bond
e.g.—$Fe^{+++}$ + kojic acid → color
c.″ Two electrovalent bonds
e.g.—$Ti^{++++}$ + maleic acid → color





A. Colored as such
e.g.—Carotene

B. Colorless (Color must be developed)
1. Oxidation
e.g.—Aniline + $PbO_2 \rightarrow$ color
2. Reduction
a. Directly
e.g.—Ascorbic acid + methylene blue → fading
b. Indirectly
e.g.—Organic peroxides + $Fe^{++} \rightarrow Fe^{+++}$
$Fe^{+++} + 6\ NCS^- \rightarrow Fe(NCS)_6^{---}$
3. Coupling
a. Directly
a.′ Inorganic reagent
e.g.—$C_6H_5OH + TiCl_4 \rightarrow C_6H_5OTiCl_3$
b.′ Organic reagent
e.g.—$C_6H_5OH$ + sulfanilic acid (diazotized) → color
b. Indirectly (intermediate compound)
e.g.—$C_6H_6 + 2\ HNO_3 \rightarrow$ 1,3-dinitrobenzene
1,3-dinitrobenzene + acetone → color

**Interferences.** Although freedom from interference by undesired constituents was mentioned as one of the requirements of an ideal color reaction, the analyst often has to deal with difficulties from the presence of interfering constituents. Brief consideration will be given to some of the ways in which the difficulty manifests itself, and to what can be done about it.

1. **Nature.** Of the various kinds of interference which are recognized, some of the most important should be noted.

The interfering constituent itself may be colored. The effect will depend upon the intensity of the extraneous color, and upon its nature.

Turbidity may develop, or a precipitate form, which will prevent accurate photometric measurement. Thus, 1,10-phenanthroline forms a color with ferrous iron and, at the same time, a precipitate with any mercuric ion present. Another kind of example is the precipitation of aluminum when one makes the solution ammoniacal prior to adding thioglycolic acid to form a color with iron.

The interfering constituent may form a color with the color-forming reagent, as does cobalt with 2,2′,2″-terpyridyl when this compound is used for forming a colored complex with iron.

Some constituents interfere by complexing the desired constituent to form an ion or compound more stable than that resulting from action of the desired constituent with the color-forming reagent.

Occasionally oxidation or reduction converts an ion into a form unsatisfactory for reaction with the color-forming reagent. Since 2,2′-bipyridyl reacts with ferrous iron, and thiocyanate with ferric iron, it is necessary in the respective cases to have the iron in the required valence state.

Another kind of difficulty results from reaction of the interfering constituent with the colored system, causing it to fade or lose its color entirely. Many reductants affect permanganate in this way.

**2. Prevention.** Since so many colored systems are subject to interference, it behooves the analyst to be alert to the possibilities, and to know what he can do to avoid the difficulty, or to reduce the effects to a negligible amount.

Undoubtedly, the most widely applicable method of avoiding interference is to follow the Biblical admonition to pluck out the offender. Translated into chemical terms, this means to separate it. Four principal kinds of methods of separation are available—volatilization, precipitation, electrodeposition, and extraction. The general operations, applications, and merits of such processes have been presented elsewhere *(145)*. Sandell *(182)* and Willard and Diehl *(233)* emphasize the problems of concentrating and/or separating traces of desired constituents. Suffice it to state here that separation, because of requirements in time, technic, and equipment, is generally an operation of last resort, something to be avoided if possible.

In some methods, such as those involving the formation of certain dithizonates, the color-forming reaction proceeds concomitantly with the separation of the desired constituent into the non-aqueous layer containing the organic reagent.

Fortunately, in many cases actual separation from an interfering constituent need not be made. Avoidance of this undesirable operation is accomplished in several ways, some of the most important of which are noted.

One of the most obvious remedies for interference is to compensate it. A familiar example is the addition to the standard solution, with which the unknown is to be compared, of an amount of the interfering constituent equal to its concentration in the known. Of course, this necessities knowing the amount of the interference. In using photometers this addition would be made to the solution in the reference beam, or to that used in preparing a calibration curve. In this way one may compensate for impurity or color in reagents. It may be



noted, incidentally, that one should not assume without test that what appears to be a colorless solvent is adequate by itself in the reference beam of the spectrophotometer.

When absorptometers are used for measurement, some interfering colors may be excluded by proper selection of filters in a filter photometer or setting of the monochromator in a spectrophotometer. The former possibility has been discussed by Knudson, Meloche, and Juday *(114)*. An example of the latter possibility is shown in the curves for manganese and chromium published by Silverthorn and Curtis *(192)*. According to this work, absorption at 575 mμ is attributed to manganese only. In either case, it is evident that the nature of the curves must be sufficiently different to make such a procedure feasible. Measurement with certain lake-forming reagents is illustrated by the work of Liebhafsky and Winslow *(125)*.

In some cases the interference must be determined and then taken into account in calculating the desired constituent. The paper by Silverthorn and Curtis illustrates the simpler of the two general possibilities. To determine the chromium, the transmittancy measurement is made at 450 mμ. Since manganese absorbs at this wavelength also, its absorptance, as determined from measurement at 575 mμ (where chromium does not interfere), must be subtracted from the total absorptance at 450 mμ. A more complicated case, involving solution of simultaneous equations, is illustrated by the work of Comar and Zscheile *(33)* on mixtures of two chlorophylls. As a general case, values for the concentration of each of $n$ components may be obtained from absorptance measurements at $n$ wavelengths, where no two curves coincide or intersect; however, no two wavelengths may be used at which absorptance values for two components are in the same proportion.

Since many reactions are affected by the pH of the solution, control of this variable offers a means of avoiding certain kinds of interference. In using 1,10-phenanthroline, for example, the pH should be within a relatively narrow range if beryllium is present. At a pH above 5.5 the hydroxide precipitates, and below 3.0 the metal complexes with the reagent. The many color formations possible with dithizone *(115)* under carefully controlled pH is a striking example of the effect of this variable.

One of the most important means of avoiding interference is to convert the interfering ion into a soluble complex, inert as far as the color-forming reaction is concerned. This requires a relatively high degree of stability in the complex. Thus, in a mixture of iron and cobalt, the ferric ion may be complexed with pyrophosphate

before adding the thiocyanate to form the complex ion $Co(NCS)_4^{--}$. To make most effective use of similar reactions, we need a comprehensive compilation of the many complexing possibilities, including tabulation of the relative stabilities of the complexes with both organic and inorganic reagents.

Control of the valence state of an ion, either the desired or interfering constituent, provides in some cases for avoiding interference. Phosphate or fluoride ions, for example, form a complex with ferric iron. In their presence the iron may be reduced to the ferrous state and then complexed with thioglycolic acid to form a suitably colored system. In such a case the reductant itself must not complex the desired constituent, as sodium formate would iron.



# CHAPTER 2

## COLOR STIMULIMETERS

Consideration will be given first to the intruments called colorimeters by physicists. Since such apparatus is designed to match, by means of a suitable combination of known stimuli, the stimulus of the system measured, the term color stimulimeter seems appropriate. All students of optics recall matching a given color by means of three rotating disks, colored red, green, or violet, and so arranged that the relative amounts of the three "primaries," or stimuli, could be varied until a match was obtained. Different modifications of such devices depend upon the nature of, and the method of combining, the stimuli *(34e)*.

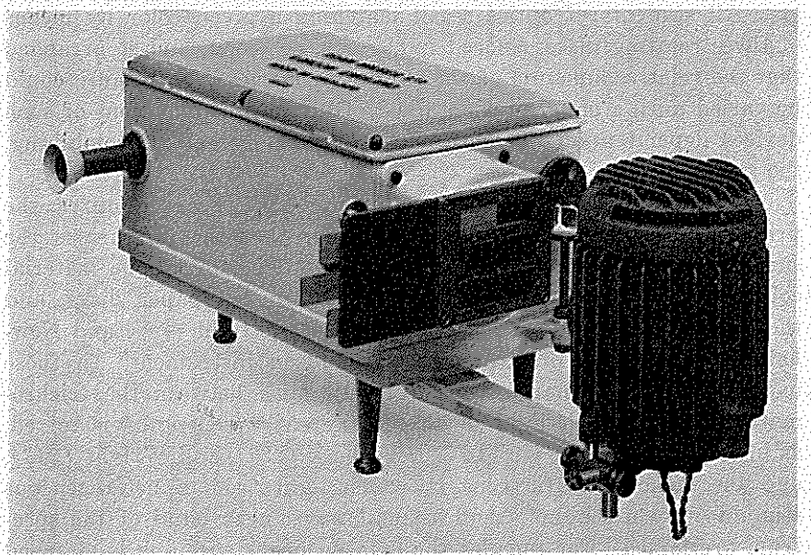
In the additive type of stimulimeter the observer mixes the primaries or standards in such a manner that the mixture is the sum of the components. Examples of those dependent upon material color standards are the apparatus using Munsell paper disks, and the instruments designed by Donaldson *(41)*, Guild *(78)*, and Newhall *(164)*. Fig. 1 illustrates the Donaldson instrument. In those using spectrum primaries we may distinguish between the trichromatic type in which light of three different wavelengths is mixed, as in the apparatus of Guild *(77)*, Verbeek *(220)*, and Wright *(243)*, and the monochromatic type in which light from a heterogeneous stimulus ("white light") and a small wavelength band of the spectrum are mixed. For purples the heterogeneous stimulus is matched by adding the spectrum light to the sample light, as in Priest's apparatus *(172)*.

In subtractive instruments light from the illuminant is passed successively through the standards, each of which in turn absorbs part of the light transmitted by the previous one, until a match is obtained. The standards may be such materials as solutions *(11)*, wedges of dyed gelatin *(104)* or glass *(106)*, or glass disks, as used in the Lovibond tintometer *(211)*.

It should be noted that any combination of stimuli found to be a visual match for a given system constitutes a colorimetric specification for the system, and not, at least directly, a statement of the amount of a desired constituent in it. Thus, the Lovibond specification for a solution containing 0.3 ml. of 0.04 per cent bromthymol blue

added to 10 ml. of a pH 7 buffer, when viewed through a 12.5 cm. glass cell in northern daylight, is given by Taub *(208)* as 0.2 red, 1.6 yellow, and 3.5 blue, which means that the color of a solution of this thickness and concentration matches the combination of the three glasses noted.

These numerical specifications are useful for production purposes in many industries in which color plays a part in the quality of the products, such as textiles, inks, paints, dyes, and pigments. Products having the desired characteristics may be taken as standards and their specifications determined. Conformity to these values may then



(Courtesy of Adam Hilger, Ltd.)

FIG. 1. Donaldson color stimulimeter.

be taken as a measure of the acceptability of subsequent lots. Permissible color tolerances must be established, of course.

Such instruments have not found wide use thus far in chemical work. In addition to the difficulty of reproducing arbitrary filters and of relating their tristimulus coefficients (See p. 106) to concentration of solute in a solution, more important difficulties have prevented general adoption of such apparatus, especially the additive type. Some of the instruments are too complicated for routine work, and in all of them the quality of the light source must be carefully maintained and the observer should have a normal visibility curve and normal mixture data *(85)*. Further information on these instruments may be found in treatises on physics and in papers by Guild *(79)*. A brief description of two English instruments is given here.



More nearly objective colorimetric specifications are being determined to an increasing extent by means of spectrophotometers *(66)*. This subject is discussed later in connection with these instruments.*

**The Lovibond Tintometer.** Since the Lovibond tintometer has found some use in America for specification work for various industrial products, especially oils, and since it has been applied in metallurgical analysis *(209)*, this instrument has been selected for description as one of two examples of stimulimeters.

The principle of the instrument is the matching of a color against the light transmitted by one or more of a set of glasses, each glass being of a slightly different tint from those on either side of it in the set. There are three series of glasses: red, yellow, and blue. The members of each series differ from each other by definite increments, and equal values of the three series, superimposed on each other, absorb white light.

Fig. 2 shows the simple instrument incorporated in a cabinet to provide reproducible lighting conditions. The quoted descriptive matter is from a pamphlet issued by the manufacturers *(211)*.

"The instrument consists of a monocular eyepiece at the end of a rectangular tube which fits into a housing containing the Lovibond color slides. Near the end of the tube remote from the eyepiece are two mirrors placed at an angle of 45 degrees to the line of sight so as to reflect the light entering the two rectangular apertures in the far side of the housing into the eyepiece. When looking through the eyepiece, these two apertures appear as one rectangular field divided in the center by a hairline.

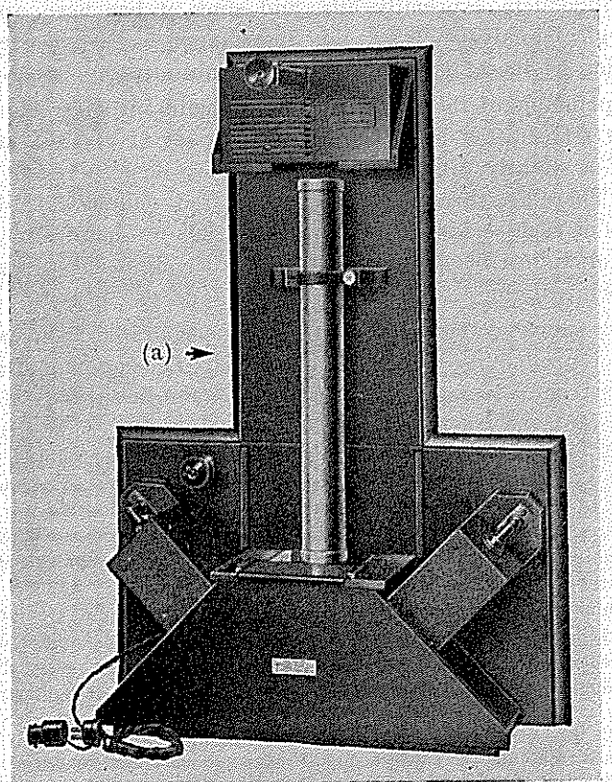
"Within the case is a series of racks controlled by triangular pointers which protrude through the slotted lid of the case. Each rack holds nine slides of the Lovibond color scale and, as the pointer is moved along its slot, every slide in the rack is brought in turn into a position to intercept the beam of light striking one

* NOTE: Two other widely used methods of attempted specification of color may be mentioned. Perhaps the earliest, and still the one most used by non-scientists, is verbal description, expressions such as "robin's egg" blue being familiar to all. The unsatisfactory status of this method is generally recognized by scientists. A much simplified, and fairly definite, verbiage, recommended by the Inter-Society Color Council *(109)*, has been adopted as official for pharmaceutical products and seems likely to find some general use.

A second method consists in preparing color standards such as glass, porcelain enamels, textiles, paper, and other materials. Dealers' paint cards and the samples of the Textile Color Card Association are well known examples. Two important systematic schemes for applying this second type should be mentioned. In the Munsell system *(34e, 107, 161)* each standard sample has a designation, such as *R* 4/10, the *R* standing for red (the hue), the 4 for value (on the brightness scale), and the 10 for chroma (on the saturation scale). In the Ostwald system *(34e, 102, 108)* each standard sample has a designation, such as 12 *ne*, the numeral standing for one of the 24 hues, and the letters for a specific mixture of the hue with white and black.

of the two mirrors. The value of the slide effective in the instrument is shown by the indicator in the rectangular opening at the end of the slot in the housing. Normally, slides value 0.1 to 0.9 are fitted into one rack of each color, slides value 1.0 to 9.0 into a second, and slides value 10.0 to 18.0 into a third.''

It has been practically impossible to convert Lovibond readings, of the kind mentioned, to the standard tristimulus system of the International Commission on Illumination (See section on Color




(Courtesy of Tintometer, Ltd.)

FIG. 2. Lovibond Tintometer arranged for long columns of liquid in vertical cells.

Analysis). Recently the instrument has been modified so that the new Lovibond-Schofield apparatus makes such conversion possible *(189)*. Fawcett *(50)* has surveyed 60 years of colorimetry involving tint meters.

Directions for making observations with either type of instrument are given in the pamphlet cited, together with a statement concerning Lovibond color nomenclature. The National Bureau of



Standards has reported on likely sources of error in using these glass standards *(69)*.

**The Donaldson "Colorimeter."** The Donaldson trichromatic stimulimeter *(93)* is a visual instrument which matches the color to be measured with a combination of known colors in known proportions. For the operation of color mixing it depends upon the use of a diffusion box into which a composite beam, consisting of amounts of red, green, and blue light, variable at will, is admitted. The diffusing box mixes the colors thoroughly, and by varying the components of the mixture the operator is able to produce a variable color of known specification which can be matched to the color under test. The instrument has the advantage over some other types that no moving parts are employed, and also that the field is not intermittent so that the instrument can be operated directly from A.C. or D.C. electric supplies.

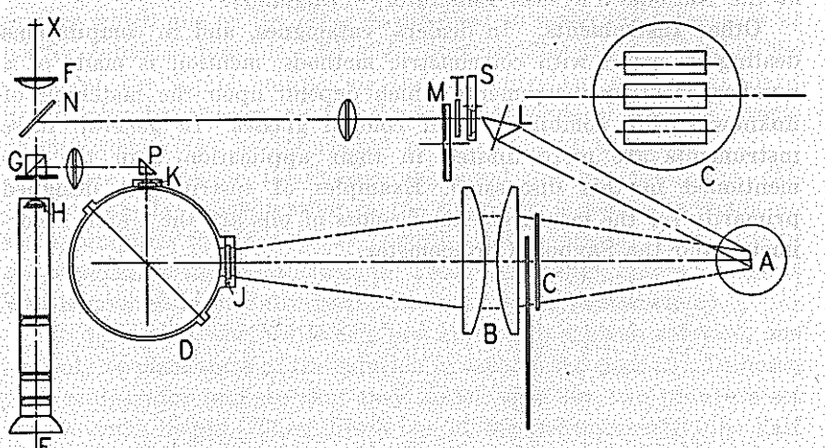



FIG. 3. Diagram of the optical system of the Donaldson stimulimeter.

"In the use of the instrument light from a lamp *A* (Fig. 3) passes through three primary color filters *C* and then through a condensing lens *B* to form an image of the light source in an aperture *J* of a hollow sphere *D*, the inner surface of which is coated with magnesium oxide and is white and diffusing. The three primary beams are mixed by diffuse reflection at the surface of the sphere. Light of uniform color emerges from a second aperture *K* in the sphere and, by means of the optical system shown, reaches the eye *E* which observes one half of the field of the photometric cube *G* filled with light from the sphere.

"The other half of the photometric field is filled with light from a specimen *X* illuminated by a standard lamp. The lenses *HF* serve to form an image of the specimen at *E* so that the aperture of the specimen is not seen by the observer when making a color match. A match is made by adjusting the amounts of the

three primary colors by means of movable shutters over the filters *C*. These shutters are controlled by knobs.

"For measurements on highly saturated colors, desaturation of the specimen color is effected with light from the source *A* reflected by the prism *L* and the transparent plate *N*. This light may pass through one of three color filters, identical in color with the primary color filters *C*, mounted on a rotating wheel *S*, or alternately through an aperture in this wheel. The intensity of this desaturating light is varied by means of a circular neutral tint optical wedge *M*. A diffusing glass *T* is situated in the course of the beam.

"Careful attention is given in the design of the instrument to the accuracy of the scales giving the amounts of the three primary colors. The primary colors of the instrument are calibrated in terms of the X, Y, and Z primaries defined by the International Commission on Illumination (See section on color analysis) so that measurements made with the instrument may be expressed in this form. They thus become independent of the particular characteristics of the instrument."

The primary color filters, consisting of mounted gelatin color screens, are stated to be quite stable. The instrument was designed at the National Physical Laboratory (England).

**Other Instruments.** In dealers' catalogues, and in compilations dealing in detail with colorimetric methods, mention is made of a number of other instruments which depend upon the matching of unknowns with combinations of colored glasses. In general, these instruments are more limited in their application than the two mentioned in this discussion. Examples of instruments designed primarily for the measurement of colors of oils are the Union Colorimeter and the Saybolt Chromometer.



# CHAPTER 3

## COLOR COMPARATORS

Many analytical determinations are accomplished colorimetrically by matching the liquid system containing the desired constituent in unknown amount with a similar system containing the desired constituent in known amount, or with something visually equivalent to such a standard. Instruments designed for facilitating the matching, and known as comparators, really enable one to determine the intensity (brightness) of the color relative to that of the standard. Many provide for viewing the standard and unknown simultaneously, during which one or the other is modified to make the intensities as nearly equal as possible. To effect the modification for liquids the depth of the liquid column or the concentration of the standard liquid may be altered, or for liquids and solids a movable, transparent wedge may be interposed to bring about a match.

If the depths or thicknesses compared are not the same, calculation of the concentration in the unknown is generally made on the assumption that the colors (depths or thicknesses) are inversely proportional to the concentrations. This relationship, known as Beer's law, is treated more fully later (See section on photometers).

To determine whether Beer's law applies to a given solution, one has only to place some of the solution in each of the cups of a Duboscq type comparator and see that they match with the two plungers set for the same height. Then dilute one of the two to exactly one half its original concentration, put it back in the cup, and match them again. The reading for this cup should now be just twice what it was before. If the law does not apply, comparison should be made in a constant depth device, or a correction should be determined, as mentioned in the discussion of Duboscq comparators.

**Properties Desired in Standards.** The nature and the characteristics of the standards for comparison are important. Where routine determinations are to be made, it is desirable to prepare the standards in permanent form, if possible. For this purpose it is generally preferable to use the desired constituent itself, and to have the unknown and standards in the same physical state. Thus, an unknown in liquid form is compared with another liquid rather than with glasses, color cards, or other dissimilar materials. In determining iron, for exam-

ple, it is convenient to keep a standardized solution of an iron salt, portions of which can be diluted for the required range of standards. Often such a series retains its reliability for some days, or even months. In other cases, in order to allow for compensation of errors arising from variability in color development and stability, it is necessary to prepare the standard at the same time and under the same conditions as the unknown.

It may be impossible or inconvenient to use the desired constituent itself for standards. Thus, in determining residual chlorine in water by means of ortho-tolidine, standard solutions of chlorine water are both difficult to prepare and unstable. For a considerable number of these cases visually equivalent (for at least one kind of illumination) standards, many of which are permanent, have been prepared from various materials. The A.P.H.A. standards for determining iron by the thiocyanate method consist of mixtures of potassium chloroplatinate and cobaltous chloride solutions. Hellige, Inc. and Tintometer, Ltd., use glass standards for numerous series. Occasionally a color card is satisfactory for rough work, as with the use of nitrazine test paper. In all such cases, even when the standard and unknown are both liquids, their spectral transmittance characteristics are seldom the same, which may render matching impossible under an illuminant having a spectral distribution different from that used in establishing the original matches. Fig. 4 shows spectrophotometric curves for the transmittancies of several solutions and their corresponding standards which were matched visually for a given illuminant.

Furthermore, dichroism *(34b, 241)* in solutions or glasses may complicate the problem of using standards different from the unknown. Thus, bromphenol blue, recommended in the Clark and Lubs series of pH indicators, is dichroic; that is, different thicknesses exhibit different hues. Corresponding inorganic standards, consisting of mixtures of solutions of colored salts would probably not show this phenomenon. In such cases, the original matching thickness must be used.

For visual comparison, whether with a comparator, a filter photometer, or a spectrophotometer, it may be best to make the match only after the eye is dark-adapted. Many consider that best work is done in a booth fitted for maintaining reproducible working conditions.

**Spectral Characteristics of the Illuminant.** The spectral distribution may differ widely for the radiant energy emitted by different



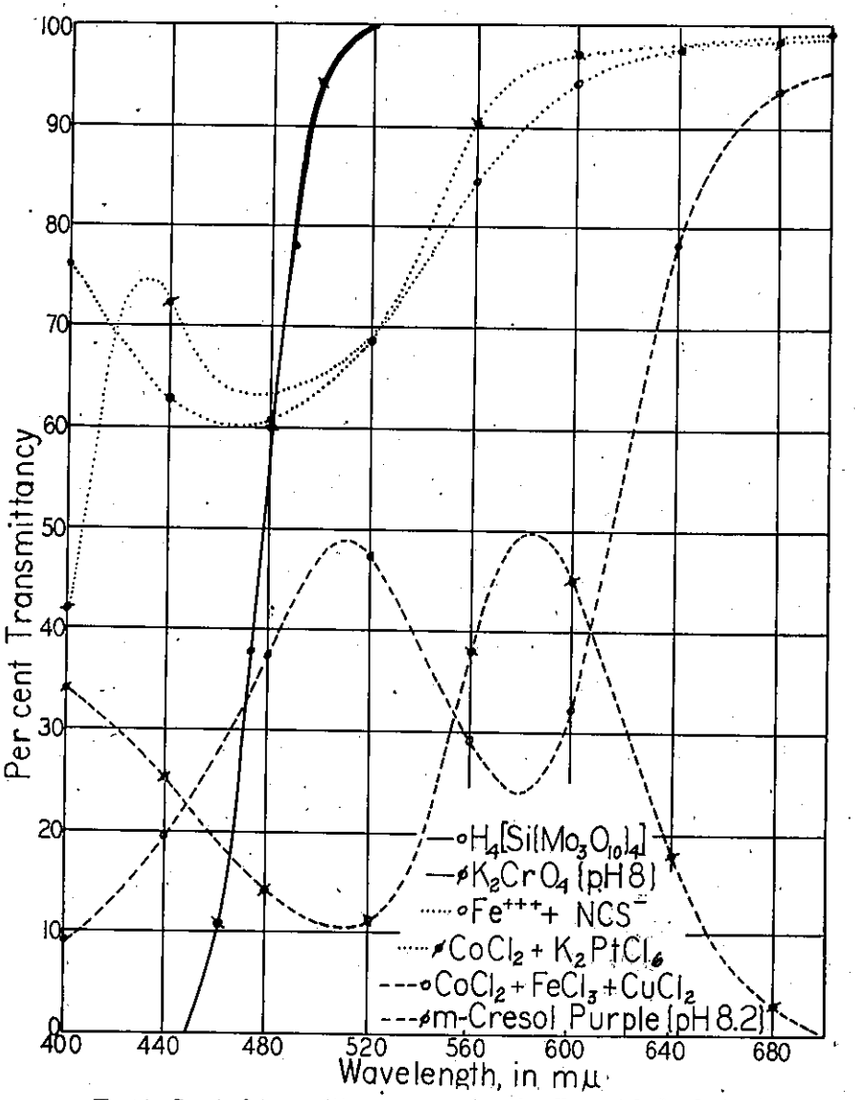



FIG. 4. Spectral transmittancy curves for visually matched solutions.

illuminants. Examples of such light sources are the sun, a tungsten incandescent filament, a mercury arc, and a neon tube. Systems matched with standards of different composition (and probably of different spectral characteristics) under one kind of illuminant may well not match under an illuminant having a different spectral energy distribution.

**Disadvantages.** Although matching methods, particularly the simplest of the standard series type, require a minimum of time and equipment for the determination, they may have at least one or more of the following disadvantages:

1. The observer may not have an adequate sense of color discrimination.

2. Unless permanent standards are satisfactory, one must take the time and material to prepare other standards, usually solutions, in some cases as often as each day.

3. As already pointed out, the general range of sensitivity is ± 5 per cent of the total amount present. For very low and very high concentrations the eye is incapable of detecting small differences in intensity. Even in the workable range the relatively low sensitivity of a method may be attributed to the fact that differences in concentration of the desired constituent in nearly matching tubes account for only a small proportion of the total light transmitted. Variations are found for different colors and different observers.

4. The presence of interfering colors practically eliminates this type of method.

5. Some types of comparators require relatively large volumes of the unknown solution.

6. Suitable illumination may not be available.

**Errors with Comparators.** In connection with the previous discussion of characteristics desired in solutions and standards and of disadvantages of comparator methods, the possibility at least of certain kinds of errors has been implied. Snells' treatise, under the heading of accuracy, summarizes a number of definite sources of error in matching methods. Specific items mentioned include general and specific limitations, mechanical errors, optical errors, reading errors, dilution errors, temperature errors, time errors, reagent errors, interfering ion errors, operator errors, turbidity and colloidal particles, dichroism, and artificial standards.

**Technic of Comparison.** Among the many devices which have been used as apparatus for comparators *(197)* four general types may be recognized. The principles and applications of these methods will now be considered.

1. **Standard Series Type.** Probably the oldest, and still the cheapest and most widely used, technic of comparison employs a series of standards. These are selected so that the range of intensities (concentrations) extends from less than to more than that of the unknown.

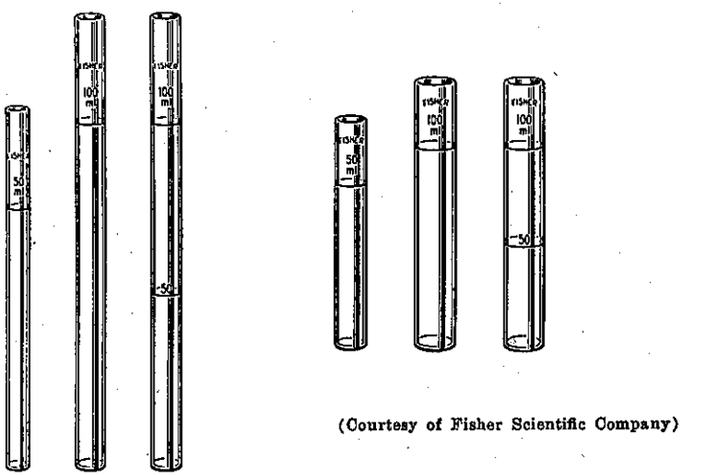


The latter is then compared with each member of the series until a match is found, and the concentration of desired constituent is assumed to be that of the corresponding standard. It is assumed, of course, that the thickness or depth of unknown and standard is the same if they are solutions.

This procedure is especially desirable for solutions not conforming to Beer's law (See section on photometers).

Solutions or glasses are used most as standards to match unknowns in solution, and the matching is nearly always accomplished visually.

a. **Comparison with Solutions.** To hold solutions a variety of forms of containers is used. For small thicknesses, such as 1 to 2 cm., test



(Courtesy of Fisher Scientific Company)

FIG. 5. Short and tall form Nessler tubes.

tubes or French square bottles of uniform thickness are suitable for horizontal observation. Greater thicknesses may be observed best vertically in Nessler tubes, made preferably with plane glass bottoms fused on. Various depths are available, in tall or short forms, graduated either for a particular volume or with many marks, like a buret. Graduated lengths of 24 or 30 cm., holding 50 to 100 ml., are usually most convenient. Fig. 5 illustrates two forms.

In matching the unknown against the standard one may simply hold two tubes side by side and look through them, preferably against some white background to reflect the light through the tubes. Greater precision and convenience is achieved by means of some optical aid, such as those described herewith.

a.′ **Comparator Blocks.** Comparator blocks are helpful if containers such as test tubes are to be observed horizontally. This device has been much used in the determination of pH values by comparison of the unknown solution with standard buffer solutions.

The form suggested by Clark *(29)* is illustrated in Fig. 6. The verticle holes, in parallel rows, should be just large enough to accommodate the test tubes, usually 5- or 6-inch, and as close together as practical. For observing the solutions the horizontal holes, about 10 mm. in diameter, should pass perpendicularly through the vertical holes. The best effect is obtained with a soft wood block having the holes blackened by means of an alcoholic wood stain.

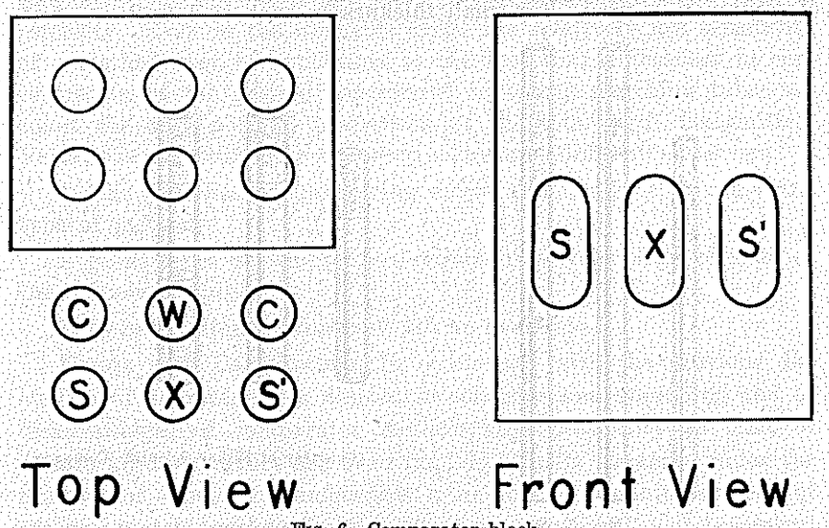
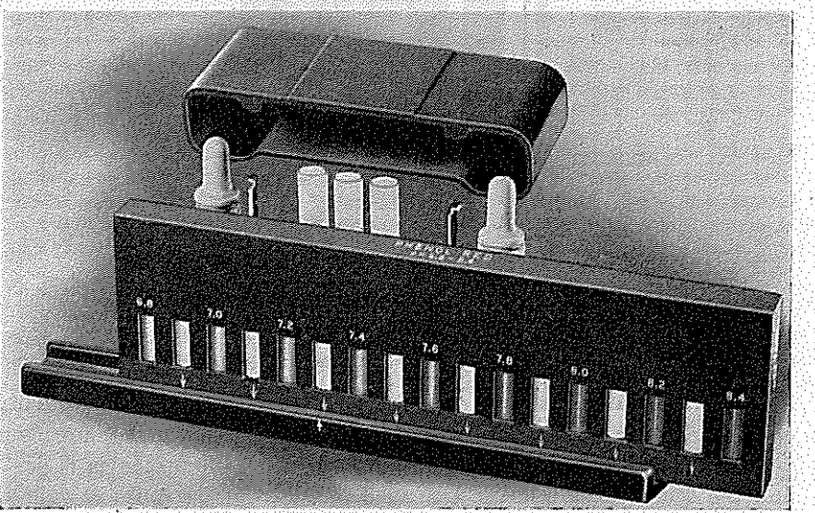



Fig. 6. Comparator block.

The block, as illustrated, may be used in several ways. If the sample is clear and colorless, and the unknown is to be compared with single standards, one uses tubes in the front row only, $X$ being the unknown and $S$ and $S'$ the two standards judged to be near it in color. Samples not too colored or turbid may be measured in this same way by superimposing the back row of tubes on the front row. The control tubes $C$ compensate the color or turbidity inherent in the unknown $X$ and the center tube of water $W$ maintains a constant thickness of liquid. A block with three rows of holes could be used for measuring colored samples by means of Gillespie's method, which is described later. The block as shown could be used for this method with clear, colorless samples.



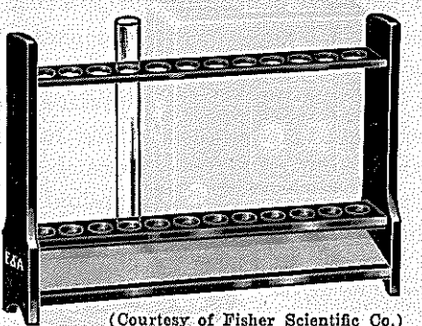
b.′ **Slide Comparators.** In order to facilitate comparison of tubes a number of devices have been proposed for moving the series of standards past the tube containing the unknown. One of the common designs is that of Taylor, illustrated in Fig. 7. A number of other types are now available for a variety of industrial determinations. Some forms are designed as roulettes.




(Courtesy of W. A. Taylor and Co.)

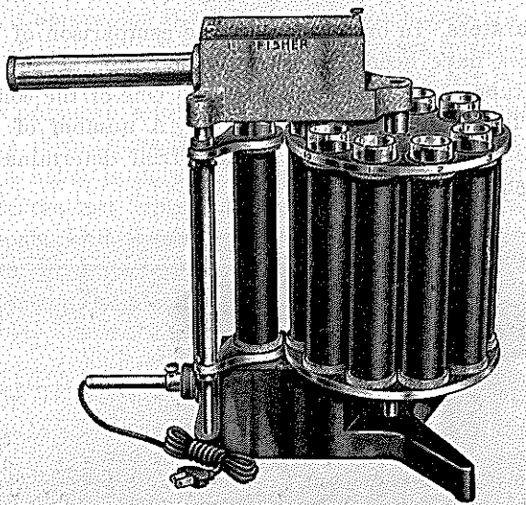
Fig. 7. Slide comparator with solutions as standards.

c.′ **Devices for Vertical Comparison.** Vertical comparison of standard series of solutions, which provides for viewing greater thicknesses, is usually accomplished in tubes of the Nessler design. Fig. 8 shows a common type of rack for holding such tubes. A mirror, near the



(Courtesy of Fisher Scientific Co.)

Fig. 8. Nessler tube rack.

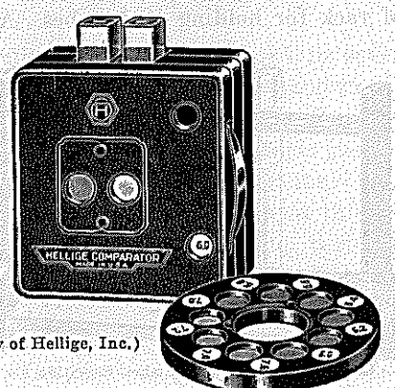


(Courtesy of Fisher Scientific Co.)

FIG. 9. Roulette Nessler tube holder.

bottom, is arranged to reflect light up through the tubes. The unknown and standards are compared by placing them adjacent to each other and looking vertically down through them. A more convenient modification is shown in Fig. 9, in which the unknown is fixed and the series of standards, arranged in the roulette rack, is rotated until a match is found. The optical scheme, taken from the Campbell-Hurley comparator, makes the light from each of the two tubes being observed illuminate half of the circular field in the eyepiece.

b. **Comparison with Glasses.** Several manufacturers sell compact comparators in which the standards are glasses fitted into a roulette




(Courtesy of Hellige, Inc.)

FIG. 10. Roulette comparator with glass standards.



disk. Fig. 10 illustrates the Hellige instrument. The following description is taken from a circular describing the similar Lovibond instrument:

"The apparatus consists of a Bakelite case opening like a book, and furnished at the back with an opal glass screen and two compartments to receive test tubes, or in certain instances rectangular cells, containing the liquid under examination. In the front portion are two circular holes situated side by side opposite the opal screen and coinciding with the vessels containing the solutions under examination.

"Bakelite disks, each fitted with colored glass standards, fit in turn into a recess in the lid of the comparator and are held in position by four spring balls which engage in a groove moulded in the edge of the disk. The disk in the comparator is thus free to revolve about its axis and each color standard in turn passes in front of the left-hand aperture.

"As the disk revolves, the value of the color standard visible in the left-hand aperture appears at the indicator recess near the right-hand corner of the comparator case. The disks which are available for numerous tests are easily inserted and withdrawn. Any initial color of the sample naturally affects the color developed (with a reagent), so provision is made for a blank or untreated sample of the test solution to be placed behind the color standard when necessary, in a vessel of the same internal diameter as that containing the sample to which the reagent is added. This arrangement automatically corrects the color standard for any color inherent in the solution under test and thus ensures that the same color standards are applicable for use with either colored, slightly turbid, or water-clear solution.

"The glass color standards with which the Bakelite disks are fitted consist of Lovibond red, yellow, and blue color slides cemented together. They are permanent in hue, saturation, and brightness."

**Determination of pH Values.** The colorimetric method for determining hydrogen ion concentration, or more strictly pH values, has been widely used. Industrial applications merit mentioning the procedure. It is based upon the action of certain indicator compounds which exhibit definite hues in solutions of given concentrations of hydrogen ions. Thus, bromthymol blue is yellow in acids at pH 6.0; as the pH value increases, the hue changes gradually to green at the neutral point, and then to blue as the solution becomes basic, deep blue being reached at pH 7.6. Certain other indicators go entirely through their hue transformation on one side or the other of pH 7.0. Since these changes of hue are reproducible for given concentrations of the indicator, to determine pH values of aqueous solutions one needs only sufficient indicators to cover the pH range to be measured and some method of comparing the colors with standards.

One interpretation of such color changes follows. These acid-base indicators are either weak acids or bases. Assume that we are dealing with the acid, HIn, and that we have the equilibria

$$\underset{\text{Unionized form}}{HIn} \rightleftarrows \underset{\text{Iogenic form}}{HIn'} \rightleftarrows \underset{\text{Ions}}{H^+ + In'^-}$$

in which the form HIn has one color (or none) and the anion $In'^-$ has another color. Then

$$\frac{[HIn']}{[HIn]} = k_1$$

$$\frac{[H^+]\,[In'^-]}{[HIn']} = k_2$$

Multiplying $$\frac{[H^+]\,[In'^-]}{[HIn]} = k_1k_2 = k_{In.}$$

$$[H^+] \cdot \frac{[In'^-]}{[HIn]} = k_{In.}$$

Thus, the hydrogen ion concentration $[H^+]$ multiplied by the ratio $[In'^-]/[HIn]$ is a constant. A change in $[H^+]$ means a change in the ratio, and, consequently, in the color.

One scheme for applying this method consists in adding a definite amount of the indicator solution to a definite volume of each of a series of solutions of known concentrations of hydrogen ions (pH values, actually), called buffer solutions. The same volume of unknown, in a tube of the same dimensions, is treated with the same amount of indicator and the solution is then compared with the knowns in order to find a match. This procedure amounts to a standard series comparison, and usually comparator blocks or slide comparators are used.

The useful indicators are of the same type as those used in determining end points in neutralization titrations. The list given in Table I is probably most useful for general work. It will be noted that each indicator covers only a limited pH range. The necessity of selecting the one including the pH value of the unknown solution may require some preliminary testing. A long-range or "universal" indicator is useful for this purpose.

The most reliable method, proposed by Clark and Lubs, requires the preparation of a series of buffer solutions having pH values 0.2 pH units apart. To make the entire series of solutions, including purifying and testing the materials, takes considerable time and work. If the requirements of the problem justify this effort, details of the procedure should be consulted in standard works. The error in careful work is usually less than 0.1 pH unit.



Such colorimetric determination of pH values may be subject to a number of sources of error, in addition to those of a general nature previously mentioned. These include at least the following: filtration of sample, contamination with acid or base, salt error, protein error, and indicator error. Details regarding these items may be found in the sources already noted or in the treatise by Snell and Snell *(196)*.

TABLE I.

*Selected pH Indicators*

| Common Name | pH Range | Color Change | pK | Concn. |
|---|---|---|---|---|
| | | | | % |
| Thymol blue | 1.2- 2.8 | red-yellow | 1.7 | 0.008 |
| Bromphenol blue | 3.0- 4.6 | yellow-lavender | 4.1 | 0.008 |
| Bromcresol green | 4.0- 5.6 | yellow-blue | 4.7 | 0.008 |
| Bromcresol purple | 5.2- 6.8 | yellow-purple | 6.3 | 0.012 |
| Bromthymol blue | 6.0- 7.6 | yellow-blue | 7.1 | 0.008 |
| Phenol red | 6.8- 8.4 | yellow-red | 7.7 | 0.004 |
| Cresol red | 7.2- 8.8 | yellow-red | 8.1 | 0.008 |
| Thymol blue | 8.0- 9.6 | yellow-blue | 8.8 | 0.008 |
| Thymolphthalein | 9.3-10.5 | colorless-blue | 9.2 | 0.008 |

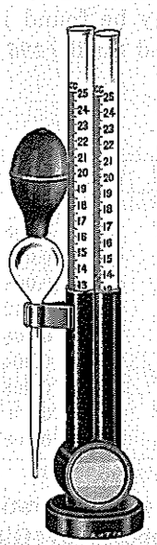
A second method, which may be considered as a milliliter-ratio modification of the Clark and Lubs method, was proposed by Gillespie *(72)* to eliminate the necessity for buffer solutions. The results obtained with it may not be quite as reliable as with carefully prepared buffer solutions, but the method is more easily carried out and it is satisfactory for many purposes. The unknown is matched with two tubes together, one containing indicator in the basic form and the other containing it in the acidic form (Ref. *145*, p. 379). Then, using the pK value in Table I

$$pH = pK + \log \frac{\text{ml. of indicator in basic form}}{\text{ml. of indicator in acidic form}}$$

**2. Duplication Type.** A simple type of comparator is based upon matching the unknown solution by duplicating its color with a known solution. Some authors refer to duplication as colorimetric titration. The unknown is placed in a suitable container, such as a Nessler tube, and the volume is brought to a given mark or depth. In a similar tube, marked for the same depth, any necessary color-forming reagents are added and nearly enough solvent to bring the volume to the mark. Then from a buret one adds carefully a standard stock solution of the desired constituent until, on mixing and bringing the volume exactly to the mark with solvent, the two tubes match. The amount of standard used gives the amount of desired constituent

in the unknown solution. As with standard series matching, the system need not follow Beer's law. Matched comparison tubes should be used. This procedure is in routine use in some steel mill laboratories.

3. **Dilution Type.** In the two kinds of methods of comparison described, Beer's law need not apply. We come now to two kinds in which it should apply, since their use is based upon the assumption that the concentration of the color-forming constituent is proportional to the depth of solution observed.



(Courtesy of A. H. Thomas Co.)

FIG. 11. Dilution comparator.

In the first of these two methods the known and unknown solutions, of different color intensities, are placed in comparison tubes of the same thickness and internal diameter, and graduated for reading the volumes of solution. In use the more concentrated solution is diluted with solvent until, on stirring and observing the tubes horizontally, the two colors appear matched. Then the concentrations are inversely proportional to the volumes (or depths) of the solutions. Closer matching may be achieved by using one of the dilution comparators available, an example of which is shown in Fig. 11.

Rodden *(179)* gives references to applications of a modification of this method in which observation is limited to certain absorption bands for salts of the rare earth elements.

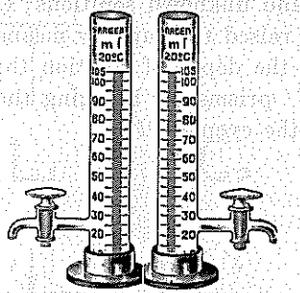
4. **Balancing Type.** Another widely used method consists in comparing the unknown solution with a single standard solution, the depth of one being fixed and that of the other changed until a match is obtained. If the systems conform to Beer's law, the concentrations of the desired constituent in the two solutions are inversely proportional to the depths of solution measured. For systems not conforming to this law the two solutions must have nearly the same concentration, or a correction must be applied as obtained from an experimentally determined calibration curve *(245)*.

Snell and Snell *(196)* and Yoe *(244)* show a number of the many variations of design of comparators used. All of these were made for visual observation. Goudsmit and Summerson *(74)* proposed an interesting modification in which the matching is accomplished by two photocells. Drabkin *(42)* recommends the use of filters (see section on filter photometers) in all Duboscq-type instruments. Brewster *(18)* used such apparatus for determinations in work with sugars.

In its simplest form this procedure requires only two similar

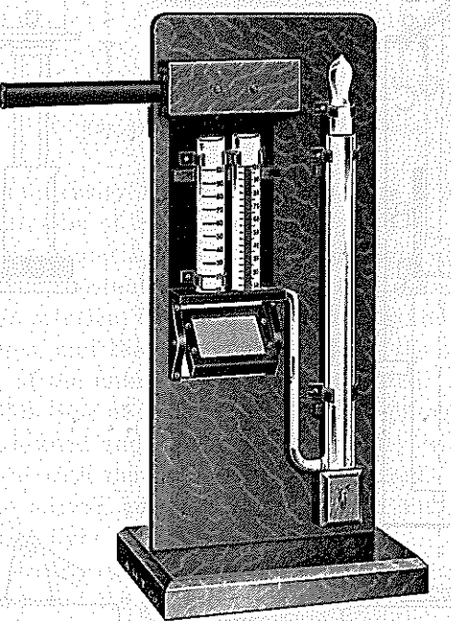


graduated cylinders. If one uses Nessler tubes, the known solution may be put in one and then the unknown in the other until the two match when observed vertically over a suitable reflecting surface. Then the volumes or depths are read and the concentration calculated. The Schreiner comparator represents a refinement of this technic. A variation of such simple technic employs two matched Hehner cylinders, illustrated in Fig. 12. In this case, having put the known and unknown solutions in the two tubes, one simply draws off liquid,



(Courtesy of E. H. Sargent and Co.)

FIG. 12. Hehner cylinders.



(Courtesy of A. H. Thomas Co.)

FIG. 13. Kennicott-Campbell-Hurley comparator.

by means of the stopcock, from the tube having the more concentrated solution until the two match. The Kennicott-Campbell-Hurley comparator, Fig. 13, employs this principle by fixing the depth of solution in one tube and varying that of the other manually.

**The Duboscq Comparator.** The best known type of instrument for making measurements by comparison is the Duboscq comparator. Fig. 14 illustrates the arrangement of the following essential parts: *A*, a mirror for directing light through the cups; *B* and *B*,′ glass bottom cups for the known and unknown solutions; *C* and *C*,′ glass prisms around which can be raised the cups by means of rack and pinions; *D* and *D*,′ to change the depth of solution through which the light passes; and *E* and *E*,′ prisms for bringing the light beams together in a divided field in the eyepiece *F*.

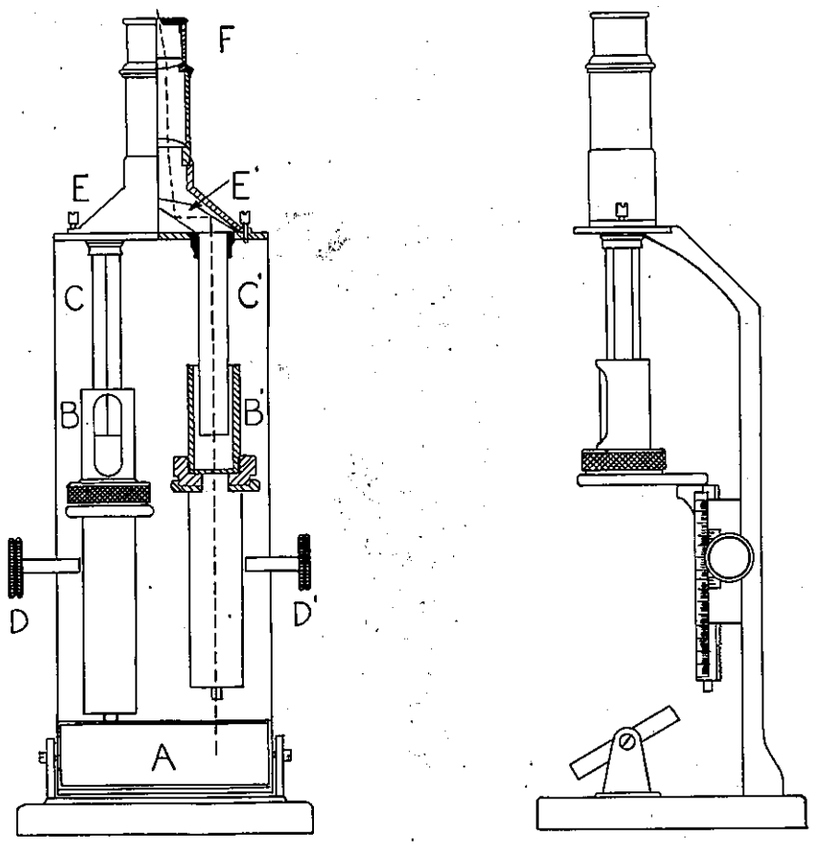



Fig. 14. Diagram of Duboscq comparator.



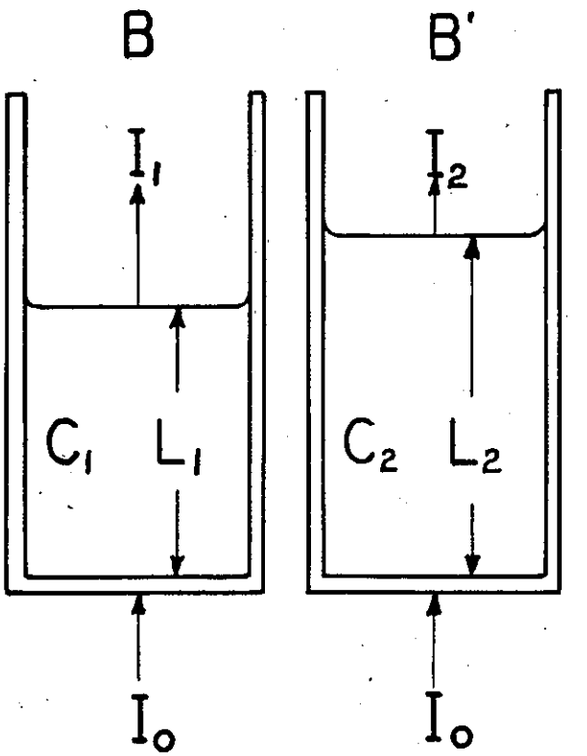



Fig. 15. Diagram of Duboscq cups.

If light of intensity $I_0$ is incident on cups $B$ and $B'$ (Fig. 15), containing solutions of concentrations $C_1$ and $C_2$, adjusted to such depths of liquid $L_1$ and $L_2$ that the emergent light intensities $I_1$ and $I_2$ are equal, $I_0$ is a constant and $I_1$ is equal to $I_2$. Assuming Beer's law applies to each solution, $C_1L_1 = C_2L_2$. Knowing the depths $L_1$ and $L_2$ in the two cups, and the concentration $C_1$ of the standard, the concentration $C_2$ of the unknown is easily calculated.

In using this type of instrument, the glass plungers or prisms and the cups are cleaned and then either wiped dry with cleaning tissue paper or rinsed with the solution to be measured. After wiping any dust from the mirror, adjustment is made to obtain the most satisfactory indirect light.

To check the instrument, some of the colored solution should be put in each cup and the plunger set in one of them for some definite depth, such as 30 mm. The other plunger is then moved up and down, approaching and passing repeatedly the point of match, nar-

rowing the range gradually until the point of best match is obtained. The second cup should then read 30 mm.

The measurement is made by filling one of the cups half full with the unknown solution and putting in the other cup about the same amount of a solution of known content similarly prepared, or one to be used as visually equivalent to the unknown—that is, a permanent standard. The prism in the known solution is set at a definite depth, and the height of the cup containing the unknown is then adjusted until a match is obtained. The concentration in the unknown equals that in the known multiplied by the factor, depth of known/depth of unknown.

In some recent instruments *(200)* the percentage of desired constituent may be read directly.

Thiel *(210)* has proposed a modified Duboscq type comparator for determining the extinction coefficient of an unknown solution by comparison with his gray solution of known extinction coefficient.



# CHAPTER 4

## COLOR ABSORPTOMETERS

### I. FILTER PHOTOMETERS*

The third class of instruments comprises devices which provide a measure of the light transmitted (or reflected) by a system, relative to that incident upon the system. Since the property measured is really the light absorptive capacity of the object illuminated, the instruments may be called absorptometers. Instead of being graduated in terms of absorptance, the conventional practice is to use transmittance (or something related to it, such as extinction coefficient) for transparent media and reflectance for opaque material. Appropriate names for the respective instruments would then be transmissimeters and reflectometers.

At this point certain terms should be defined. Although a number of them are not commonly used in chemistry, they have application in general colorimetry. The definitions are adapted from the reports of the Committee on Spectrophotometry *(68)* and of the new Committee on Colorimetry *(34c, d)* of the Optical Society of America.

**Laws of Absorption.** Experimental study of the absorption of light by homogeneous, transparent solids and solutions led to the formulation of the two laws stated in Tables II and III, relationships of much importance in chemical colorimetry. Since photometers are used to determine the magnitude of absorptance, it is appropriate at this point to consider briefly the significance of these generalizations.

a. **Bouguer's Law.** The first of these laws, formulated by Bouguer, expresses the relationship between absorptance and thickness of the absorbing medium. *Each layer of equal thickness absorbs an equal fraction of the light which traverses it.* The intensity of the emitted light decreases exponentially as the thickness of the absorbing medium increases arithmetically; that is, the absorption varies directly as the logarithm of the thickness. *There are no known exceptions to this law for homogeneous systems.*

* NOTE: See Die Chemie 55, 361 (1942) and 56, 183 (1943) for abstracts of some 25 lectures on the principles and recent applications of analytical colorimetry and photometry presented at a symposium of the analytical group of the Verein deutscher Chemiker.

TABLE II.

*Terms Relating to the Rectilinear Transmission of Homogeneous Radiant Energy Through a Homogeneous, Isotropic, Non-Metallic Medium in the Form of a Plate with Plane, Polished, Parallel Surfaces Perpendicular to the Direction of Propagation.*

Let $b$ = distance between the bounding surfaces
$E_1$ = radiant energy incident on the first surface
$E'$ = radiant energy reflected at the first surface
$E_I$ = radiant energy transmitted by the first surface
$E_2$ = radiant energy incident on the second surface
$E''$ = radiant energy reflected at the second surface
$E_{II}$ = radiant energy transmitted by the second surface

Then $R = E'/E_1 = E''/E_2$ = reflectance
$T' = E_{II}/E_1$ = external (over-all) transmittance *
$T = E_2/E_I$ = internal transmittance
$t = T^{1/b}$ = transmissivity **
$D = -\log_{10} T$ = optical density
$k = -\log_{10} t$ = transmissive index

* NOTE: In the spectrophotometry report this is designated transmission. Now words ending in -ion are reserved for process terms. Much use has been made of the word transmission to denote a general phenomenon.
** NOTE: This relation is known as Bouguer's law (incorrectly as Lambert's). Obviously for unit thickness, $t$ is transmittance.

TABLE III.

*Terms Relating to a Substance in Homogeneous Solution in a Solvent Contained in a Cell with Plane, Parallel Sides Perpendicular to the Direction of Propagation, the Propagation Through the Cell and the Solution Being Rectilinear.*

Let $T'_{sol.}$ = transmittance of a given cell containing solution
$T'_{sov.}$ = transmittance of the same, or duplicate, cell containing pure solvent

Then $\mathrm{T} = \dfrac{T'_{sol.}}{T'_{sov.}} = \dfrac{T_{sol.}}{T_{sov.}}$ * = transmittancy (100T = percentage transmittancy)

$\mathrm{t} = \mathrm{T}^{1/bc}$ = specific transmissivity **

where $c$ = concentration of the solution
$b$ = thickness of the solution

$\mathrm{k} = -\log_{10} \mathrm{t}$ = specific transmissive index ***

$\mathrm{bck} = -\log_{10} \mathrm{T} = \log_{10} \dfrac{1}{\mathrm{T}}$ = optical density ****

NOTES:
* Chemists generally express this ratio as $I/I_0$, $I$ and $I_0$ being respectively the intensities of the emergent and incident light.
** This relation, known as Beer's law, is only approximate in many cases.
*** This term is generally designated as the specific extinction coefficient, or absorption index. The constant k becomes the molecular extinction coefficient $K$ when the concentration is expressed in terms of moles per liter and the thickness is 1 cm.
**** This is a measure of the depth of intensity of color. Sometimes it is called absorbancy *(86)*.



In Fig. 16 let $I_0$ and $I$ be respectively the intensities of the radiant energy incident upon, and emergent from, the solution $S$, of concentration $c$, and thickness $b$.* If a layer of unit thickness transmits a fraction $t$ of the light incident upon it, a thickness $b$ will transmit the fraction $t^b$. Then

$$I = I_0 t^b **$$

This law may be expressed *(83)* in the form, $T = e^{-k'b}$, in which $T$ is the transmittancy $(I/I_0)$, $e$ is the base of the natural system of loga-

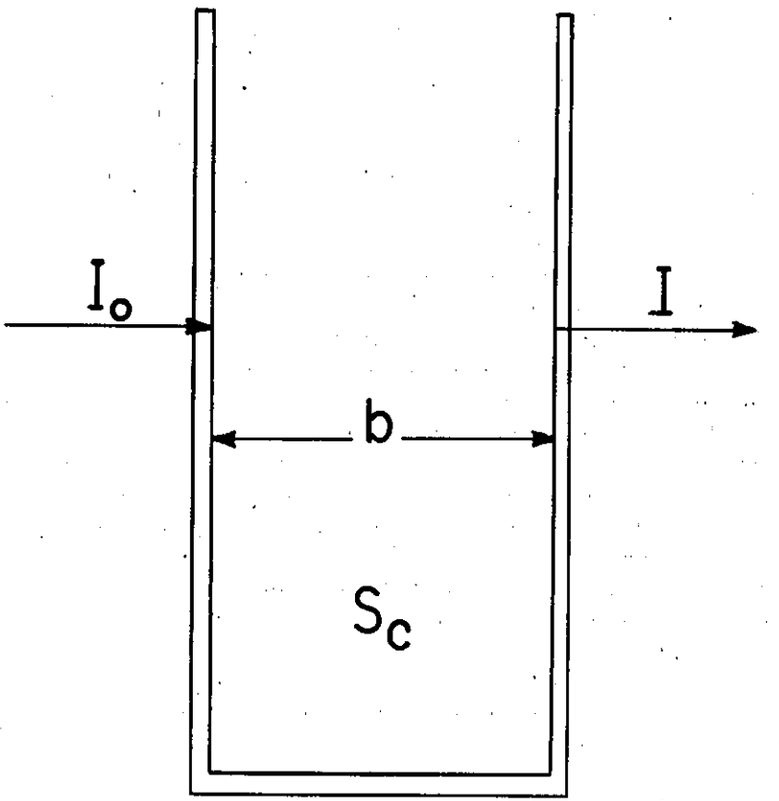



FIG. 16. Transmission of light through absorption cell.

* NOTE: The absorptance of the cell and reflectances at the surfaces are disregarded since in practice their effects are compensated by standardizing under working conditions.

** NOTE: As shown in Table III, the Spectrophotometry Committee (O.S.A.) used boldface letters for transmittancy expressions. Since the new Colorimetry Committee (O.S.A.) has not followed this usage, the author has not attempted to distinguish in this way between transmittance and transmittancy.

rithms, and $k'$, which varies with the wavelength of the incident light, is the absorption coefficient ($-\log_e t$).

A special color slide rule*** enables one to calculate easily from a known transmittancy and thickness to an unknown transmittancy at a different thickness. For example, values may be desired for 30-cm. Nessler tubes, but the apparatus available permits the determination of data for thicknesses only up to 5 cm. However, as pointed out by Hardy (*83*, p. 24), this calculation can not be made for transmittance (e.g., glass) without correction for the losses at the air-glass interfaces, each of which causes a loss of approximately 4 per cent.

b. **Beer's Law.** The second law, formulated by Beer, expresses the relationship between absorptance and the concentration of the solute in a solution. According to this generalization, the absorptance of a solution is directly proportional to the concentration of the solute (number of absorbing molecules of the absorbing substance.) Then, if $c$ is the concentration, the transmittancy $T$ for a given thickness is

$$T = t^c$$

where $t$ is the transmittancy for a solution of the same thickness having unit concentration.

This law may be expected to apply if the light is approximately monochromatic, and if the nature of the absorbing molecules is the same in solutions of different concentrations. It must be kept in mind that the absorption coefficient, $k'$, depends upon the wavelength of the incident light. Also any action in the solution, such as ionization, association, or dissociation, which affects the nature of the absorbing molecules will affect the absorption.* The change in color resulting from dilution of a solution of the dichromate ion with water is a familiar example. The equilibrium involving this color transformation may be represented by the equation,

$$Cr_2O_7^{--} + H_2O \rightleftharpoons 2\ HCrO_4^- \rightleftarrows 2\ H^+ + 2\ CrO_4^{--}$$

**Bouguer-Beer Relationships.** One may combine Bouguer's and Beer's laws for thickness $b$ and concentration $c$

$$T = t^{bc}$$

$t$ being the transmittancy for a system of unit concentration and thickness. This expression may be written

$$T = e^{-k'bc}$$

Since $T = I/I_0$

$$I = I_0 \cdot e^{-k'bc}$$

*** NOTE: Manufactured by the Keuffel and Esser Company.

* NOTE: For a more extensive discussion of these and other factors, see Ref. 116a, p. 62-83.



For unit thickness

$$\log_e \frac{I}{I_0} = -k'c = \log_e T$$

$$\log_{10} \frac{I}{I_0} = \log_{10} T = -kc$$

$$\log_e \frac{I_0}{I} = k'c = \log_e 1/T$$

For thickness $b$

$$\log_{10} \frac{I_0}{I} = kbc = D \text{ (optical density)}$$

$$c = \frac{\log_{10} I_0/I}{kb}$$

In applying these relationships in analytical determinations $b$ is known and $D$ may be determined (actually $I/I_0$ is generally measured). Then one may calculate $K$ or $k$ for a system of known concentration.* Knowing $k$, $D$, and $b$ for an unknown solution, its concentration may be calculated, on the assumption, of course, that Beer's law applies.

Solutions conforming to Beer's law show a constant molecular extinction coefficient at all dilutions and thicknesses for any given wavelength. On plotting $\log I/I_0$ ($= \log T$), or $\log I_0/I$ on an equal division axis, or $T$ on a logarithmic axis, against concentration a straight line indicates such conformity (See Fig. 45).

The molecular extinction coefficient, $K$, should be determined for the wavelength of maximum absorptance of the system, since at this wavelength there is the largest change in the constant for a given change in concentration. This gives a curve having the best slope (See Fig. 45).

Examination of these relationships shows that the measured transmittancy is a function of the absorptive capacity of the system, the cell length, and the concentration, and that the numerical value of the extinction coefficient depends on the units used in expressing $b$ and $c$. The literature is very confusing on this point, and Drabkin *(42)* has compiled a table of the many terms and symbols which have been used. Since the new report of the Committee on Colorimetry *(34)* restricts the use of words ending in -ion to processes, the word extinction alone seems unjustified. In the past it has been applied to

* NOTE: Some writers use $\log_e$ and then refer *(61, 96)* to the constant k' as the absorption coefficient. When $\log_{10}$ is used the constant $k$ is designated as the extinction coefficient.

$\log_{10} I_0/I$, to $\log_{10} 1/T$, and to whatever values result from different units and magnitudes given to $b$ and $c$.

The author suggests using $K$ (or possibly $Mk$) for the molecular extinction coefficient—that is, $c$ is expressed in moles per liter, and $b$ is expressed in centimeters. Following Brode *(21)*, $k$ would be the specific extinction coefficient, if $c$ is 1 gm./$L$., and $b$ is 1 cm. Then $D$, the optical density (designated by many as simply extinction, $E$) would be used generally to apply to $\log_{10} I_0/I$ for whatever conditions of concentration and cell length happened to be used (but specified, of course) for a given measurement. The word extinctance (extinctancy), although not mentioned by the Committee on Colorimetry, seems appropriate as an alternative term for optical density.

To facilitate practical analytical work, especially the comparison of methods, Wernimont *(227)* has proposed using what may be called a specific extinctance concentration, $E_c$, which may be defined as the concentration of solute, in p.p.m. or micrograms per milliliter, required to give the extinctance (optical density) a value of 1.00 for a 1 cm. cell. Sometimes in the writer's laboratory *(238)* the sensitivity of methods has been compared in terms of the concentration (p.p.m.) required to give a transmittancy of 50 per cent for a 1 cm. cell.

**Instruments.** In general, photometers are designed to measure intensity (brightness) of illumination. Those used in colorimetry measure the proportion of light incident on a system that is transmitted (or reflected). The proportion of light absorbed by a solution depends upon the amount of the absorbing material present. In making chemical determinations by this means the analyst's problem is to relate the concentration of the desired constituent to the amount of light transmitted. Ordinarily this method is applied to homogeneous, liquid systems.

The solutes in colored solutions absorb light in certain definite regions or bands* in the visible spectrum.** The variation in transmittancy with concentration is greatest when the incident light is restricted to the spectral region of the solute's greatest absorptance. Thus, in Fig. 17 the spectral transmittancy curves for different concentrations of an aqueous solution of iron plus 1,10-phenanthroline

* NOTE: Colorless systems may absorb radiant energy in the ultraviolet and (or) infrared region, and analytical methods are based on this phenomenon. Such procedures do not seem to belong with colorimetric methods unless one adopts the little used British conception of invisible color. Some designate all such methods as absorption spectroscopy.

** NOTE: Only part of the band may appear in the visible region, the remainder being in the ultraviolet or infrared.



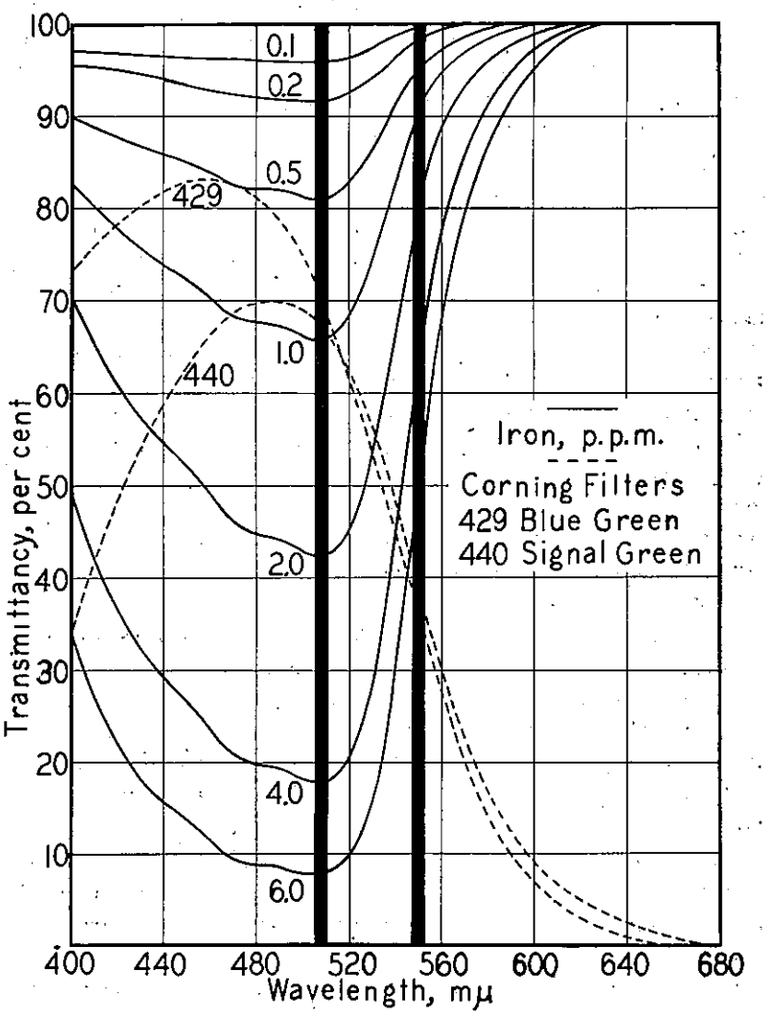



FIG. 17. Transmittancy curves for solutions of the 1,10-phenanthroline-iron complex and transmittance curves for glass filters (1 cm. absorption cells).

are shown (solid lines), along with two 5-mμ spectral bands, one centered at 508 mμ and one at 550 mμ.*** Since the difference in transmittancy between any two concentrations is much greater for the band at 508 mμ than at 550 mμ, it is obvious that greater sensitivity can be achieved by limiting the incident light to such a region.

*** NOTE: The author is not implying that a spectrophotometer set for 5 mμ isolates a band having the over-all dimensions represented. For a discussion of the characteristics of such bands, see Refs. *34b* (p. 212) and *97*.

In filter photometers this limitation is accomplished, at least partially, by interposing a suitable filter, usually of glass, between the illuminant and the observer; hence the name, filter photometer. The broken lines in Fig. 17 show the transmittance curves for two such glass filters, each 5 mm. thick. It will be noted that they pass a relatively wide spectral band of light. Selection of the best one for a given determination should be based on the spectral transmittancy curve of the solution to be measured. Thus, the absorption band of the iron-phenanthroline solution, shown in Fig. 17, nearly corresponds with the region of maximum transmittance of a signal green (Corning No. 440) or a blue-green (Corning No. 429) glass. Several manufacturers have selected a series of 8 to 10 glasses, for which the regions of maximum transmittance are fairly well spaced from 450 to 650 mμ, and usually they recommend a specific glass for a given determination. In order to narrow the band of light passed, composite filters are available consisting of two or more filtering media. Drabkin *(42)* has discussed the problems and possibilities of such combinations. Usually composite filters greatly decrease the intensity of the light beam.

Non-conformity of a solution to Beer's law may result from an unsymmetrical relationship of the transmittancy curves of the solution measured and of the filter, accompanied perhaps by difficulty of visual matching *(12)*. A self-filtering effect of the absorbing medium has been suggested *(234)* as the reason some solutions show nonconformity to the linear relationship of a logarithmic calibration curve.

As already noted, the filter should transmit the region of the absorption band of the unknown and as little as possible of the surrounding spectrum. If the spectral transmittancy curve for the unknown is not available, the best of a series of filters is the one giving the greatest difference in reading between two concentrations of the unknown solution. A general idea of the filter to use is indicated in Table IV.

When a measurement justifies its use, a monochromatic illuminant, such as a particular line of the mercury arc, may be used with such instruments. Besides the special equipment required, there may be difficulty in finding a line of the desired wavelength of sufficient intensity.

The actual quantity measured depends upon the instrument. Some are designed to give directly the percentage of incident light transmitted; others have arbitrary scales, or read in terms of some units



which must be converted to the amount of desired constituent. One of the latest announced is calibrated in terms of extinction coefficients. In some cases it may be feasible to have a scale which reads directly in terms of some one constituent for a given set of experimental conditions.

TABLE IV.

*Suitable Filter Colors* *

| Filter Color | Solution Color |
|---|---|
| Green | Purple |
| Blue to blue-green | Orange to red |
| Blue | Yellow |
| Purple | Violet or red |
| Red | Blue |

* NOTE: See the following catalogues for the spectral characteristics of glass filters: Corning Glass Works, "Glass Color Filters," and Jena Glass Works, "Jena Colored Optical Filter Glasses." Transmittance data are available in International Critical Tables and certain smaller handbooks of physical constants.

For all such instruments it is possible, and for those having arbitrary scales it is necessary, to construct a curve, from a series of standard solutions, which coordinates concentration of the desired constituent and the corresponding reading of the instrument. Fig. 18

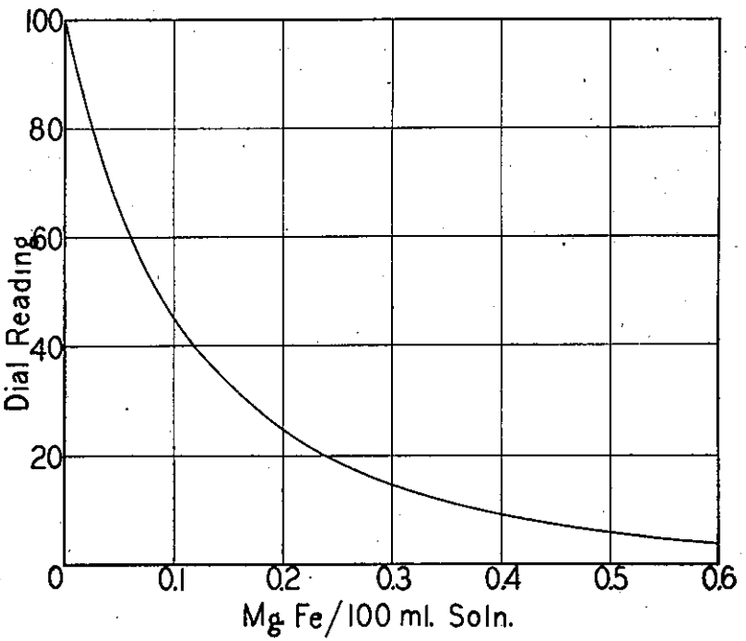



FIG. 18. Photometer calibration curve.

shows such a curve for determining iron with a home-made photoelectric instrument. In determining the concentration of an unknown solution, one simply obtains the reading on the instrument, locates the point on the curve corresponding to this value, and then reads the concentration on the other axis.

It has been noted that, for solutions conforming to Beer's law, the concentration $c$ of the solute is related to the transmittancy $T$ by the expressions

$$\log_{10} T = -kc = \log_{10} I/I_0$$
$$\text{and } \log_{10} I_0/I = kc = D \text{ (or } E)$$

These relationships are shown graphically in Fig. 19. Since these curves are straight lines originating at a known point on the ordinate axis, it is necessary to determine only a single point, on a solution of known concentration, in order to construct them.

With some instruments one has to determine the photocell response separately for $I$ and $I_0$. Others read directly in terms of $D$ (optical density) or $T$ (transmittancy). The latter type is very convenient if the values are plotted on semi-logarithmic paper, as shown in Fig. 40. Instrumental details differ in the means employed for determining $T$. If the photocell response is proportional to light intensity and the scale values range from 0 to 100, the indicator may be set at 100 with the absorption cell containing solvent only in the light beam. On placing an interchangeable, or the same, absorption cell containing the solution in the beam, the reading is the transmittancy directly, which is the ordinate value.

Once such working curves are established by either procedure, there is no further use for standards. This assumes, of course, that the experimental conditions, including the response of the photocell, remain constant. States and Anderson *(201)* recommend occasional experimental checking of such curves because of variable scattered light effects.

Neither filter photometers nor comparators yield a fundamental color specification, since they do not really measure color as such nor provide data for calculating color stimuli. These instruments probably come nearest to measuring luminance. For certain solutions Keane and Brice *(111)* proposed determining a "color index" with their instrument from the formula 100—100$G/R$, $G$ and $R$ being the measured transmittancies of the solution for the respective filters. A "color index," in terms of photometer readings, has been proposed by Diller *et al. (40)* for petroleum products.

Although some writers have referred to filter photometers as spectrophotometers, such an instrument at best can be considered as



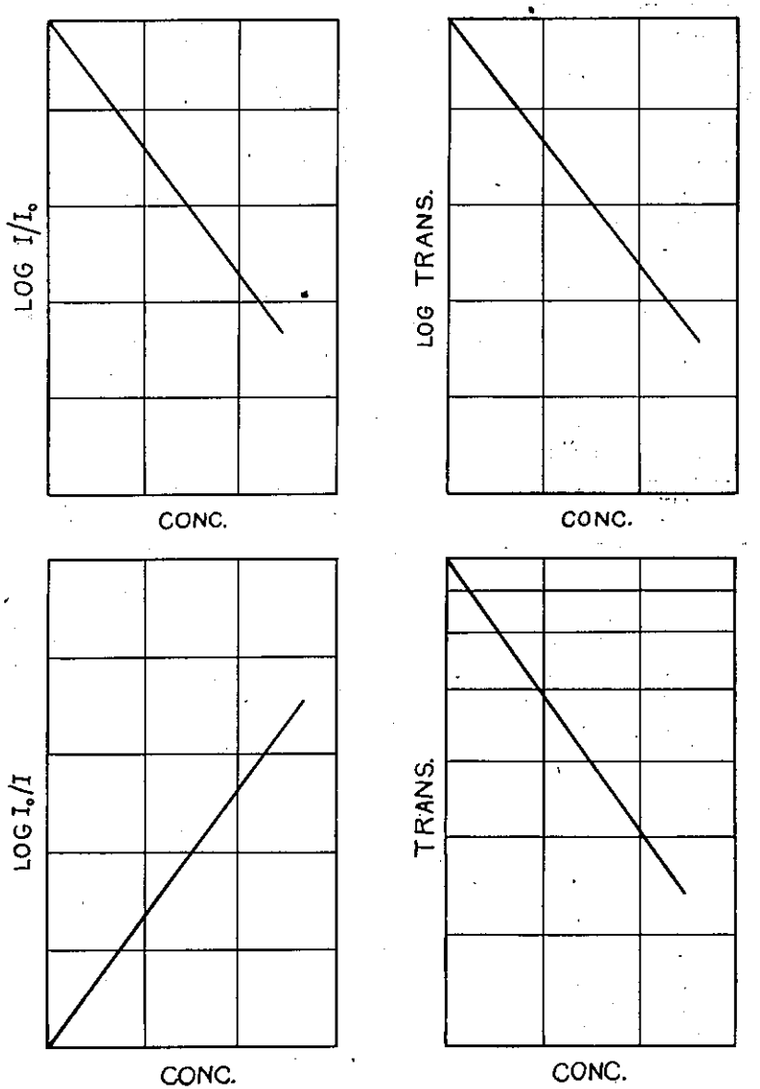



FIG. 19. Straight line calibration curves.

nothing more than an abridged spectrophotometer. It seems preferable not to use the term for such simple instruments, as lack of further specification may lead to unjustified inference regarding the reliability of reported data. If there are eight filters, for example, the transmittancy of the sample may be determined for each one. On account of the width of the spectral band passed by each filter, these eight points can not establish a reliable curve for systems having steep absorption bands. The broken line in Fig. 36 is drawn through the points obtained on such an instrument (data provided by the manufacturer), the wavelengths being those given by the manufacturer as the "medium" wavelengths of the filters. Another curve gives the data obtained for the same sample with a spectrophotometer set for a spectral band width of 5 m$\mu$.

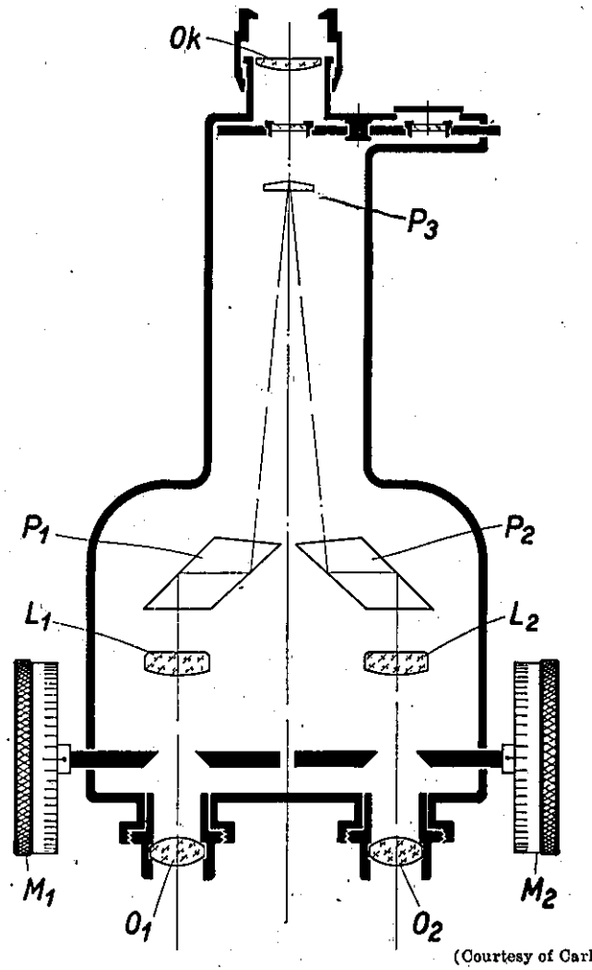
Two colored constituents together complicate colorimetric measurements. Determination with comparators is ordinarily impossible unless the amount of one constituent is known so that its effect may be compensated in the reference cell. Filter photometers do not simplify the problem much except in the rare cases where the effect of one constituent can be removed by a suitable filter. The problem of applying such photometers to a two-component color system has been discussed by Knudson, Meloche, and Juday *(114)*.

The absorption cell is used in various ways. If an arbitrary calibration curve is constructed, only one cell is needed, and it may have any usable dimensions as long as it is always used in the same way for both known and unknown solutions. When the transmittancy is to be reported, preferably the cells should have optically plane faces and a definite thickness, such as 1, 2, or 5 cm. Either the solvent and solution are measured separately in the same cell, or two optically interchangeable cells may be employed, one for the solvent and one for the solution. When using two cells, the one containing the solvent should contain the same materials as the other cell except the color-forming constituent, unless it is known that plain solvent gives the same value.

a. **Visual Type.** During recent years many colorimetric determinations have been based upon the use of visual filter photometers. The Pulfrich instrument, manufactured by Carl Zeiss, Inc. *(174)*, became available first and is best known, especially by the names gradation- or stupho-photometer. In it two light beams enter the optical system, one passing through the solvent only and the other through the solution. The observer brings the two halves of the optical field to a match by reducing mechanically the intensity of the

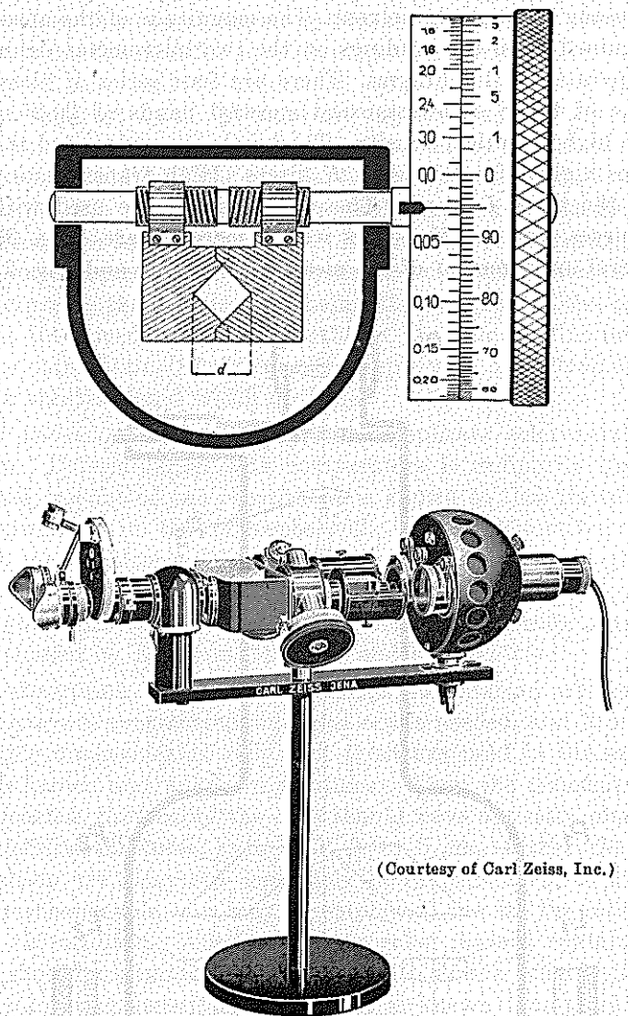


beam passing through the solvent. The magnitude of the absorptance is determined from the readings on the two micrometer drum heads which operate the diaphragms. There are four series of glass filters. The nine of the *S* series transmit spectral bands of 20-25 m$\mu$ and have the highest optical density. The bands are wider, and the transmittances higher, for the seven *K* filters. High transmittances characterize the three *L* filters. These three series are used with white light sources, and the *Hg* series with a mercury arc. Fig. 20 shows the general arrangement of the measuring system, and Fig. 21 shows




(Courtesy of Carl Zeiss, Inc.)

FIG. 20. Optical system of Pulfrich photometer.



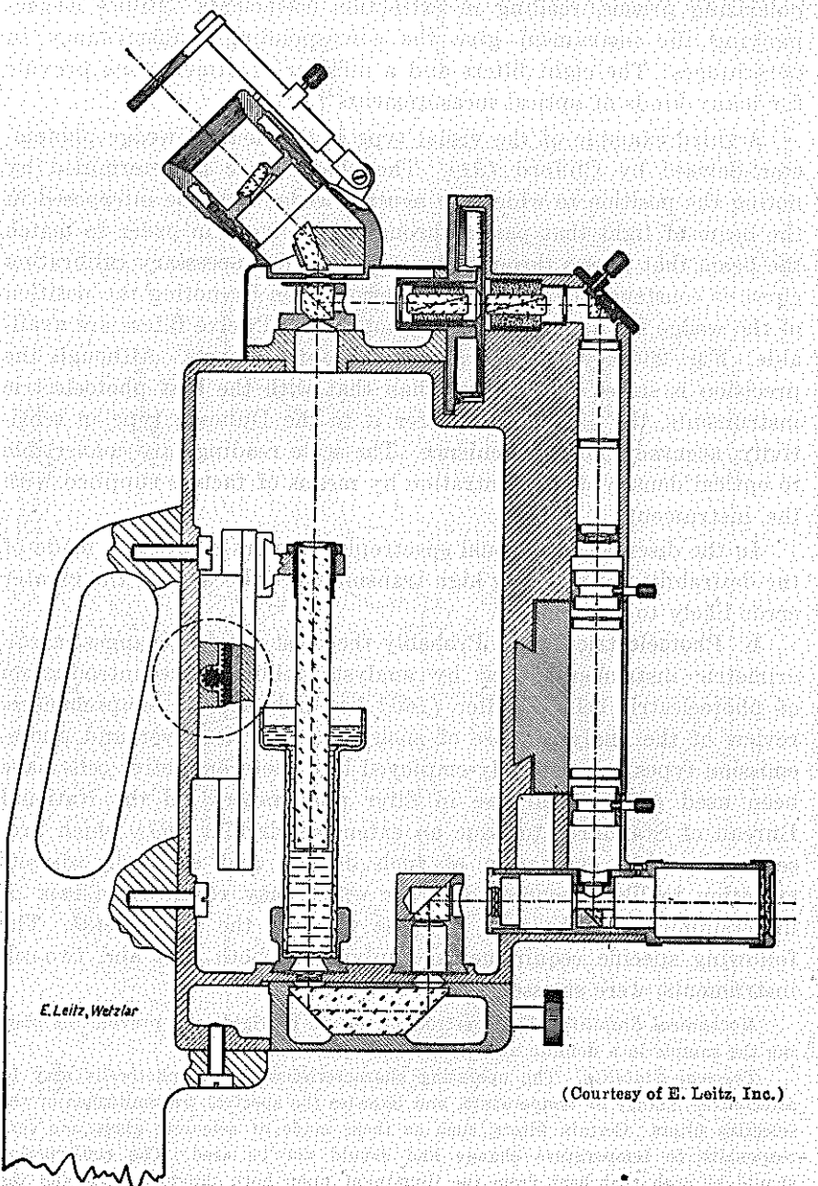

(Courtesy of Carl Zeiss, Inc.)

FIG. 21. Measuring drum of Pulfrich photometer and arrangement for absorptance measurement.

the horizontal arrangement of the instrument for measuring solutions. Many papers, in addition to a book *(118)*, have described the use of this instrument as a means of determining concentration of solutions, and for a variety of other optical purposes.

A more recent instrument of the same general type, illustrated in Fig. 22, was introduced by E. Leitz, Inc., as the Leifo photometer.






(Courtesy of E. Leitz, Inc.)

FIG. 22. Optical diagram of Leifo photometer arranged for measuring liquids.

The relative intensity of the two light beams is varied by rotation of polarizing prisms, reading in extinction coefficients. Tables accompanying the instrument give the corresponding transmittancy in percentages. The eight filters and a number of attachments provide for many kinds of optical measurements *(123)*.

A third example of the visual type is the neutral wedge photometer devised by Clifford *(31)*. The absorptance is determined by noting the position to which the neutral wedge must be interposed in the beam of light that passes through the solvent in order to match the beam that passes through the solution. The necessary calibration curve is constructed for a given determination by noting the position of the wedge for a series of known solutions. Twelve filters are available. Fig. 23 shows a diagram of the optical parts. Although the precision is stated to be lower than that with the best photoelectric instruments, the originators prefer it to the Duboscq type in sensitivity, accuracy, and convenience. The scale readings are convertible to optical density or concentration by means of factors supplied with the instrument.

In the discussion of visual spectrophotometers mention is made of the desirability of avoiding high transmittances because of the greater error likely to be involved.

b. **Photoelectric Type.** Probably the most notable changes in colorimetric instruments used by analysts followed the introduction of photoelectric cells. Müller *(160)* has published a comprehensive review of the analytical use of such cells. Barrier-layer and photoemission types are generally employed but photoconductive cells have been used *(47)*. Their use in filter photometers led the National Bureau of Standards to issue an extensive circular *(63)* which presents a critical evaluation of such devices and especially directs attention to likely sources of error which may result from lack of understanding on the part of those who use the instruments. The following specific requirements, applying to both one- and two-cell instruments, were stressed by the Bureau:

*Mechanical Stability.* The construction should be rigid, and provide for placing the sample in a definite and reproducible position.

*Thermal Stability.* The operating characteristics of the photocells may be affected by change of temperature, and likewise the spectral transmittance of the selective filters. Certain filters, such as those made of selenium glass, are very responsive to temperature change and should not be used. The construction should be such that heat from the illuminant upon both the photocells and the filters, either by radiation or convection, is reduced to a minimum.

*Electrical Characteristics.* There should be stability in the response. Certain photocells are somewhat unstable, and different readings may be obtained depend-



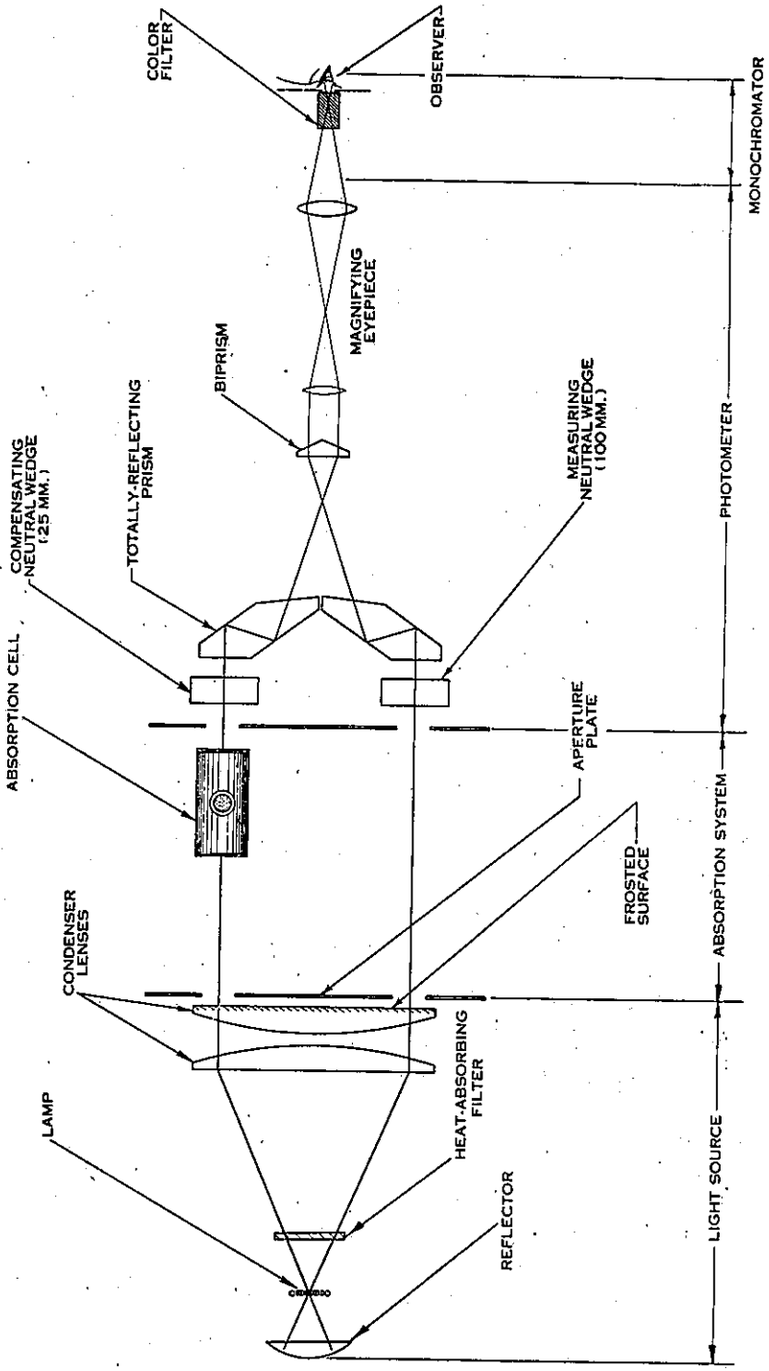



Fig. 23. Schematic diagram showing plan view of Aminco neutral wedge photometer.

ing upon whether the cell has just previously been exposed to strong or weak irradiation.

The best electrical circuit should be used *(19)*. The important question is, if the incident radiant energy is increased or decreased by any definite amount, its spectral distribution being unchanged, will the instrument reading be increased or decreased in precisely the same proportion? Under certain conditions the failure of the proportionality may be serious. The makers of cells give information on these points and their descriptive circulars should be consulted.

The response characteristics of the cells should be permanent. Some cells deteriorate so that their response to a given illumination may be appreciably lower at the end of a year than it was at the beginning. If a meter is calibrated to register a definite value for a given illumination, it is particularly important to know that the cell being used is stable in its response. In instruments where the cell response for the unknown is compared periodically with its response for known samples, or where ratios only are determined, this deterioration factor is automatically compensated.

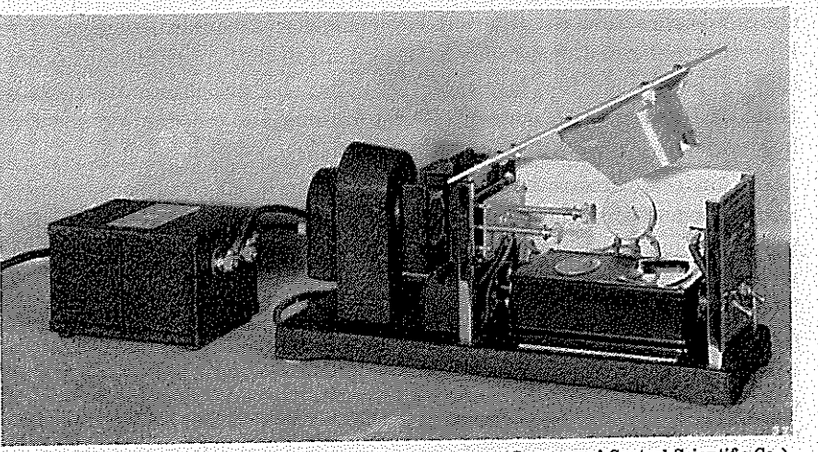
*Optical Characteristics.* In any measurement of absorptance it is important that the light pass through the solution approximately at right angles to the end surfaces of the material. Otherwise the values obtained apply to greater than the measured thickness. Furthermore it is important in accurate work to insure that the multiple reflections between the sample and the optical parts of the instrument do not introduce error in the results.

The importance of adjusting conditions to bring the measured concentration of desired constituent into a range favorable for minimizing errors is emphasized by Hamilton *(82)*. In general, the transmittancy reading should be between 5 and 90 per cent, the optimum being about 37 per cent. This conclusion applies to spectrophotometers also *(215)*. Based on determinations with Lange's filter photometer *(121)*, Schleicher has presented data *(187)* relating to the evaluation of colorimetric methods.

Many variations are found in the electrical and optical details of the designs which have been proposed. A few of these differences are noted in connection with specific examples cited herewith. Although the general tendency has been to make the instruments with arbitrary scales, a few are designed to read transmittancy, extinction, or amount of desired constituent directly. For convenience they may be considered on the basis of the number of photocells used.

a.' **One-Cell Instruments.** When only one photocell is used, the essential parts of the arrangement of most instruments consist of a light source, a container for the sample, a photocell to receive the transmitted light, and some means of measuring the response of the photocell. At present probably all of them include provision for the use of selective filters. Fig. 24 shows both the general appearance and the schematic arrangement of parts for Sheard and Sanford's design, one of the earlier instruments *(183)*. Among similar instruments





(Courtesy of Central Scientific Co.)

FIG. 24A. Filter photometer with one photovoltaic cell (showing side removed).


*Schematic diagram of the "Photelometer". The parts are: T-Constant Voltage Transformer; S-Light Source; I-Iris Diaphragm; L-Lens; A-Absorption Cell; F-Light Filter; P-Photoelectric Cell; and M-Microammeter.* (Courtesy of Central Scientific Co.)

FIG. 24B. Relationship of parts in photometer shown in Fig. 24A.

of this type are those of Diller *(39)*, Evelyn *(48)*, the Fisher Scientific Co. *(54)*, Kudor *(120)*, E. Leitz, Inc. *(124)* Müller *(159)*, and Yoe and Crumpler *(246)*.

Wood *(235)* has discussed the limitations of photovoltaic cells, which are so generally used in this type of instrument. The cell's low internal resistance results in a high output, thus eliminating need for amplification. Since the cell responds best with high-level illumination, dilute solutions are preferable. Time must be allowed for the high initial response to drop to a lower, steady level. The spectral sensitivity curve is low in the blue region, as is the energy emissive value for the filament in the tungsten lamp.

Drabkin *(42)* recommends a photoemissive one-cell instrument. There is some choice in this type of cell as regards their response

characteristics toward different wavelengths of radiant energy. The high internal resistance renders amplification both possible and necessary. Then, since low levels of light may be measured, it is easily feasible to work with concentrated solutions and/or narrow spectral band widths.

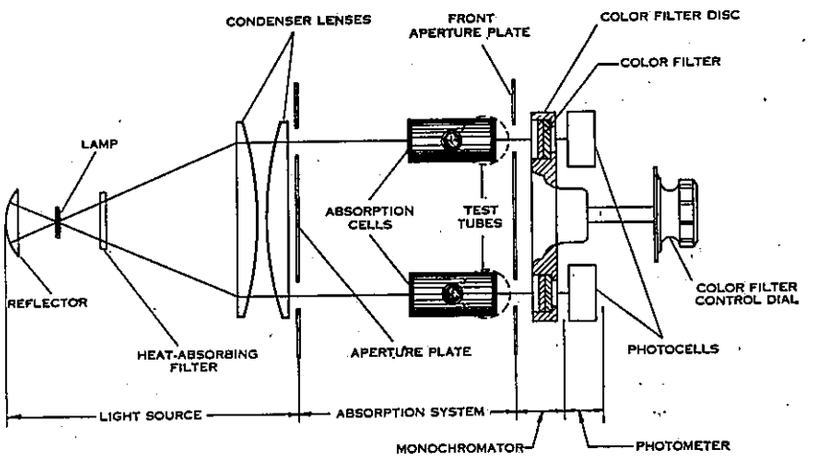
In order to insure constancy in the illuminant, and consequently in the response of the photocell (assuming that it possesses satisfactory response characteristics), the electric current for the illuminant is provided by a storage battery or a constant power transformer. The response of the photocell is detected by means of a sensitive galvanometer or a microammeter. These response readings may be the output of the cell in milliamperes or they may be arbitrary scale readings marking the position of a diaphragm or shutter, or of an electrical resistance necessary to maintain the output of the cell at some definite value. Although it is generally necessary to calibrate an instrument for a given determination by correlating concentration of the desired constituent with microammeter or other readings, a scale may be incorporated in instruments properly designed reading directly in some given terms. Thus, Kudor designed his instrument to turn a different direct-reading scale into view for each of a number of constituents to be determined. The new Leitz instrument *(124)* reads directly in extinction units, and certain others give the transmittance.

b.' **Two-Cell Instruments.** In order to avoid the provisions necessary to insure constancy of operating current for the light source in one-cell instruments, investigators rather early proposed two-cell arrangements based on the idea that fluctuations would affect the two cells equally and thus be compensated. Also the null point method of balancing the cells against each other, as indicated by a galvanometer, is supposed largely to eliminate errors arising from temperature changes or cell fatigue. Generally it is recommended that the two photocells for such assemblies be selected on the basis of similarity in spectral response. If possible, they should be nearly matched in this respect. Müller *(160)* has cautioned that two cells do not necessarily assure reliability.

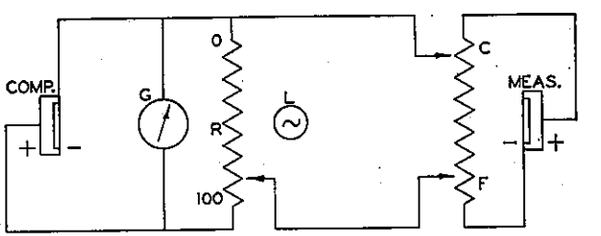
One type of arrangement for two barrier layer photocells is illustrated in Fig. 25. The essential differences from one-cell arrangements are that two light beams come from the illuminant, one going to each photocell, and that the response meter, in this case a galvanometer, is used as a null point indicator. The ordinary alternating electric current serves for the illuminant.



The use of these photovoltaic cells in this way has become quite common, representative recent apparatus of this type being described in a number of articles and manufacturers' technical publications *(3, 55, 92, 111, 121, 127, 128, 203)*. In such instruments the two photocells may be used either in a series-opposing or a parallel connection. Brice *(19)* has reviewed various proposals for such circuits and given critical analysis of the arrangements and performance of one modification. Hatfield and Phillips *(87)* have described an instrument modified for the use of Nessler tube absorption cells. The author's students use a photometer constructed according to the proposal of Wilcox *(231)*, modified to include the circuit recommended by Brice and to provide Aklo (Corning) glass filters to absorb the infrared energy from the illuminant.




**Schematic Diagram Showing Plan View of Aminco Type F Photometer**




**Circuit Diagram of Type F Photometer**

(Courtesy of American Instrument Co.)

FIG. 25. Filter photometer with two photovoltaic cells.

Some use has been made of photoemission cells. In addition to the mention made of them by Drabkin *(42)*, instruments of this type have been described by Exton *(49)*, by Mcfarlan, Reddie, and Merrill *(132)*, and by Withrow, Shrewsbury, and Kraybill *(234)*. These three contain two photocells. The last authors, whose instrument is known as the "KWSZ" photometer, stress the electrical problems involved, including what they consider the merits of photoemission cells. A modified "KWSZ" instrument has been described by Bates *(15)*.

The instrument advertised by the Eimer and Amend Company *(47)* contains two photoconductive selenium cells.

For some years there has been available the Moll colorimeter *(150)* in which sensitive thermopiles are used instead of photocells. Recently Willard and Ayers *(232)* proposed a modified thermoelectric absorptometer based upon the same general principle.

Certain modifications of this type of instrument make it possible to measure transmittancy directly by using two optically interchangeable absorption cells simultaneously, one containing the solvent only in one beam and one containing the solution in the other beam, as shown in Fig. 25. Of course, one cell may be used by substitution, as in one-cell instruments.

**Absorption Cells.** Cells used for holding liquids for work in the visible spectrum preferably should be of optical glass, with parallel faces fused on. Cemented cells may not have their specified internal thickness and the faces may not be parallel. In addition, some cements will not stand acids, bases, or organic solvents. Most parallel-face cells are made in 1, 2, or 5 cm. thicknesses, but other sizes are available *(4)*. No generalization can be made concerning the best size to use. Some instruments accommodate only one size. Where a choice is possible, the selection should depend at least upon the intensity of the color of the solution, the working concentration of desired constituent, and the amount of solution available. A 5-cm. cell may be preferable for a weakly colored solution, of which the supply is adequate. In other cases a very thin cell is required. For general conditions in spectrophotometry it is preferable to control conditions so that most of the spectral transmittance curve will lie between 10 and 90 per cent.

A number of the cheaper instruments among the filter photometers use less carefully made cells, some being nothing more than test tubes. Ordinarily, if such a container is broken, the instrument should be recalibrated for the new tube on account of the question of



interchangeability. In all cases the cells should be tested to insure that they are of high enough quality to provide the required accuracy. Precautions may have to be taken to place non-uniform test tubes in the same position each time for instruments with such equipment. Optical glass cells should be handled carefully to avoid marring the faces.

Most filter photometers have appeared since 1925. At present both visual and photoelectric instruments are well known. On account of their differences in construction, it is convenient to consider the two types separately.

**Analytical Applications.** The general analytical utility of filter photometers is well illustrated in the publications by Sandell *(182)*, Vaughan *(219)*, and Haywood and Wood *(88)*, the last two of which deal with the determination of various elements in metallurgical materials. As another specific example, Hoffman's book *(95)* deals with clinical methods, as used in medical and biochemical laboratories. In general, it seems probable that any method usable by comparimetric procedures can be adapted to filter photometers.

**Fluorimeters.** Certain substances, which are not colored themselves or do not react with color-forming reagents, show color when irradiated with ultraviolet radiant energy of suitable wavelength. The phenomenon is known as fluorescence.

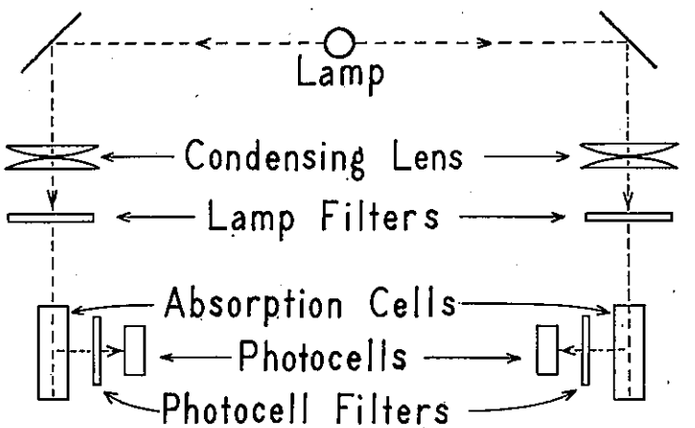



FIG. 26. Schematic arrangement of two-cell fluorimeter.

Fig. 26 illustrates the general arrangement of a 2-cell filter photometer *(110)* arranged for colorimetric measurement of such systems. Compared to the conventional filter photometer, the following important differences may be noted (a) the source of radiant

energy is usually a mercury arc lamp, certain lines of which provide the ultraviolet radiant energy; (b) the lamp filters pass only this ultraviolet radiant energy; (c) the photocell filters pass only visible radiant energy; and (d) the radiant energy measured emerges from the absorption cell in a direction at right angles to that of the incident beam.

Although fluorescing solutions are in general not as useful analytically as the ordinary colored systems, there are important applications, such as those for aluminum *(230)*, riboflavin *(94)*, and thiochrome *(90)*. The book by Radley and Grant *(177)* gives a general survey of the subject.



# CHAPTER 5

## COLOR ABSORPTOMETERS

### II. SPECTROPHOTOMETERS*

Spectrophotometers, like filter photometers, are instruments for determining the proportion of light incident upon a body that is transmitted or reflected by it. The former are distinguished by the complexity of the equipment, and especially by the inclusion of a monochromator which enables the operator to make the measurement at any desired average wavelength and with any desired width of spectral band down to the limit of satisfactory operation. The monochromator may be considered as a very refined type of filter. Although spectrophotometers have been known for many years, it is only recently that their value in analytical work has begun to be adequately appreciated.

As with other kinds of color measurement, this discussion is limited to work in the visible region of the spectrum. A general review of such apparatus was made some years ago by a committee of the Optical Society of America *(68)*. Various other sources may be consulted for a discussion of the subject from particular viewpoints *(21, 42, 62, 65, 85, 117, 122, 154, 214, 225)*.

Essentially a spectrophotometer consists of a light source for illuminating the sample, a monochromator for isolation of the desired spectral band for the illumination, a means for measuring the unabsorbed light, and a holder for the sample. Variations in all of these items, found in different instruments, depend upon the principle of construction and the purpose and requirements to be met in use.

Occasionally a monochromatic light source is used, such as a mercury arc. This limits the readings to the wavelengths of the lines available. The National Bureau of Standards is equipped to operate its visual König-Martens instrument in this way. The alternative is to use an illuminant with a spectral distribution covering the whole region to be measured, such as incandescent projection lamps having ribbon filaments. In photoelectric instruments these lamps must be operated under as nearly constant conditions as possible.

* NOTE: Since photometry deals with the measurement of light *(63)*, spectrophotometry is photometry as a function of wavelength or frequency.

Prisms or gratings serve to disperse the heterogeneous radiant energy ("white light") into its spectral components. The monochromator provides for selection of the particular region or band desired. The beam from a double monochromator is more nearly monochromatic, the effect of stray light being largely eliminated thereby. Also, the narrower the slits on the monochromator, the more nearly monochromatic is the beam. Fig. 36 shows the difference in the curves obtained with an expensive filter photometer and with spectrophotometers operating on spectral band widths of 5 or 35 mμ. For narrower slit widths the difference is even more marked. This indicates the necessity for writers to state specifically the conditions under which a curve has been obtained, especially in view of some tendency to call a filter photometer a spectrophotometer.

The method of measuring the unabsorbed light varies with different types of instruments. In a number of them there are two light beams of equal intensity from the illuminant. One illuminates the standard and the other the unknown. In reflection work the standard is usually some material such as magnesium oxide or carbonate. In transmission work it is convenient to use optically interchangeable absorption cells, the one in the standard beam containing the solvent only and the one in the unknown beam containing the solution. Thus the effect of cells and solvent cancel out. Since the solution absorbs more light than the solvent, the emergent beams have unequal intensity. By means of a photometer in the light path the more intense beam may be reduced to match the other, as determined by bringing the two halves of an optical field in a visual instrument to equal intensity. The graduation of the instrument enables one to determine the proportion of the light incident on the solution that is transmitted by it. The ratio is the transmittancy. Multiplying by 100 gives the percentage transmittancy. Some instruments are graduated in terms of optical density (or extinction), or of the angular position of the prism in a polarizing photometer.

With single beam, photoelectric instruments the usual practice is to determine the swing of a galvanometer for the solvent and the solution separately. Generally the average of several readings for each is taken, and the transmittancy obtained from the ratio of the two averages. The tediousness of the operation is obvious. This practice of averaging a series of readings is even more necessary for visual instruments. With some photoelectric instruments the scale may be set for 100 per cent for the solvent only, and then the scale reading with the solution in the beam is the percentage transmittancy directly.

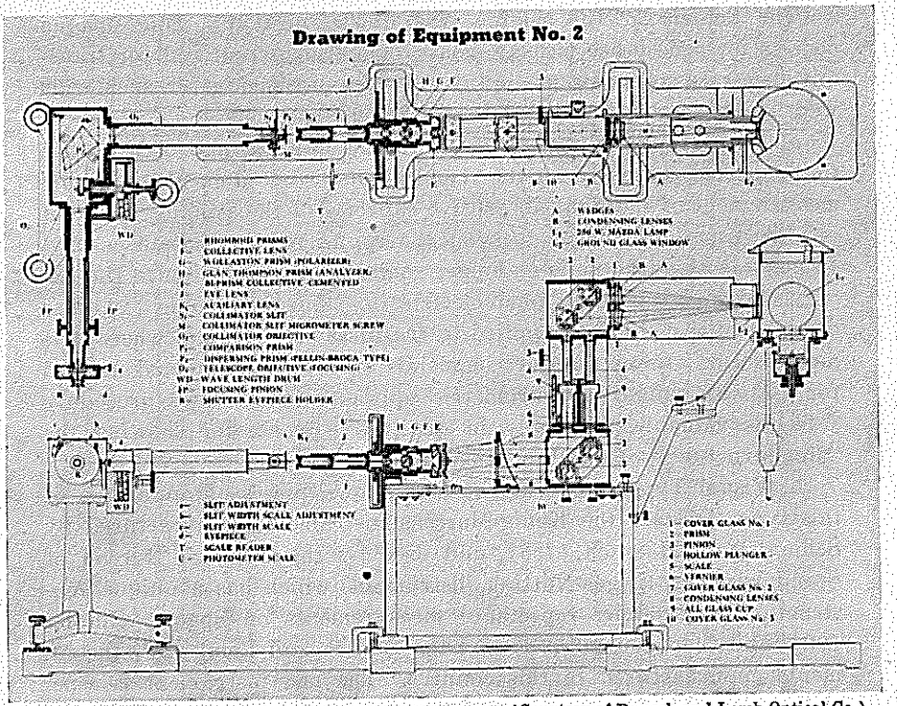


The holder for the sample is a device for supporting opaque objects for reflectance measurements, or a transparent cell for transmittance measurements of liquids. The requirements for such cells were stated under the discussion of filter photometers.

a. **Photographic Type.** Some of the older work, and occasionally that reported currently for the visual region, is based on photographic measurement of the light absorptive capacity of the sample. The film or plate functions as a photochemical receptor for the radiant energy. In general, this technic is no longer common in comparison to the use of visual and especially photoelectric instruments. Brode *(21)* has described the method as applied to the ultraviolet region, where it is most useful. Owens *(166)* recommends an internal standard method for use with emissive spectrometric equipment. Holiday's procedure *(97a)* is valuable, especially in qualitative work, for showing the "fine structure" of an absorption band.

b. **Visual Type.** Four of the best known pieces of apparatus of this type *(64)* are the König-Martens instrument, manufactured by Schmidt and Haensch *(188)*, and used by the National Bureau of Standards; the Hilger-Nutting instrument, which has a unique construction for making reflectance measurements on opaque samples *(90)*; and the Gaertner *(59)* and the Bausch and Lomb *(16)* instruments, manufactured in this country. The last instrument is especially adaptable for a variety of transmittancy measurements if the different attachments are available. Fig. 27 is a diagram of the optical details, and Fig. 28 shows it fitted with cells for variable depths of liquid. All the better instruments have variable calibrated slits for controlling the width of the spectral band of light used. The cheaper Keuffel and Esser instrument *(112)* is of interest in having a rotating sector photometer, as compared to the polarization photometer used in most other instruments. Various details of operation for the König-Martens instrument are given by McNicholas *(133)*. This publication contains a valuable discussion of general sources of error.

Visual instruments have two serious defects: (a) the physical fatigue that accompanies making a large number of observations; and (b) the limited spectral range in which data of satisfactory reliability are obtainable. The latter difficulty may be predicted from the form of the relative luminosity curve which shows the low sensitivity of the human eye below 430 or above 680 mμ (See Fig. 48, curve $\bar{y}$). In these two regions one may secure a few reliable points by opening the slits and using monochromatic light sources, such as particular lines of the mercury arc light or of certain gas tubes. Also these instruments require considerable time. Often one needs




(Courtesy of Bausch and Lomb Optical Co.)

FIG. 27. Schematic diagram showing arrangement of optical parts in the Bausch and Lomb spectrophotometer fitted with variable depth absorption cells.

points at some 30 wavelengths for a curve extending across the visible region, and usually a reliable single value may necessitate averaging five readings.

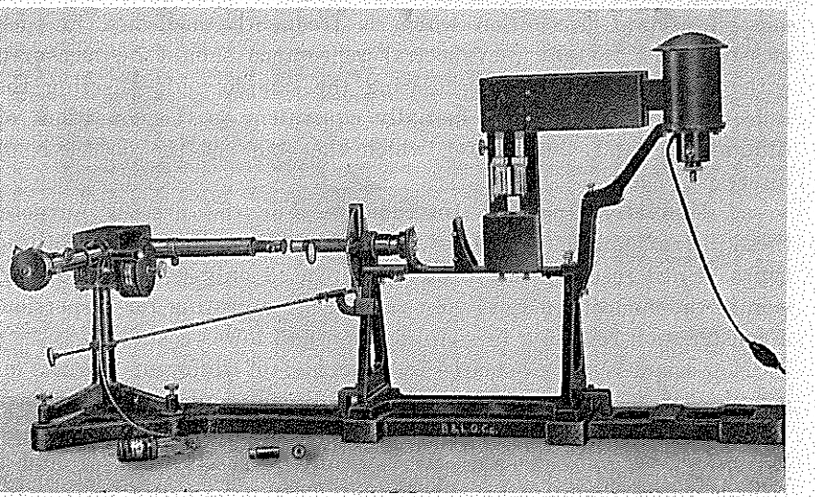
Drabkin *(42)* has directed attention to the dependence of the percentage error of visual readings of transmittancy on the magnitude of the transmittancy value. High values give the greatest errors.

Details regarding construction and operation of the instruments may be obtained from the manufacturers' technical bulletins.

c. **Photoelectric Type.** On account of these limitations of visual instruments, many efforts have been made, especially since 1925, to substitute a photoelectric cell for the human eye as the receptor for the radiant energy. Early difficulties with photocells have been overcome until certain instruments so equipped are now very satisfactory.

Fig. 29 indicates 72 possible kinds of such spectrophotometers *(101)*, considering only the major variations that can be used in the spectral, geometrical, and photometric parts of the instruments. In





(Courtesy of Bausch and Lomb Optical Co.)

FIG. 28. Bausch and Lomb visual spectrophotometer with variable depth absorption cells.

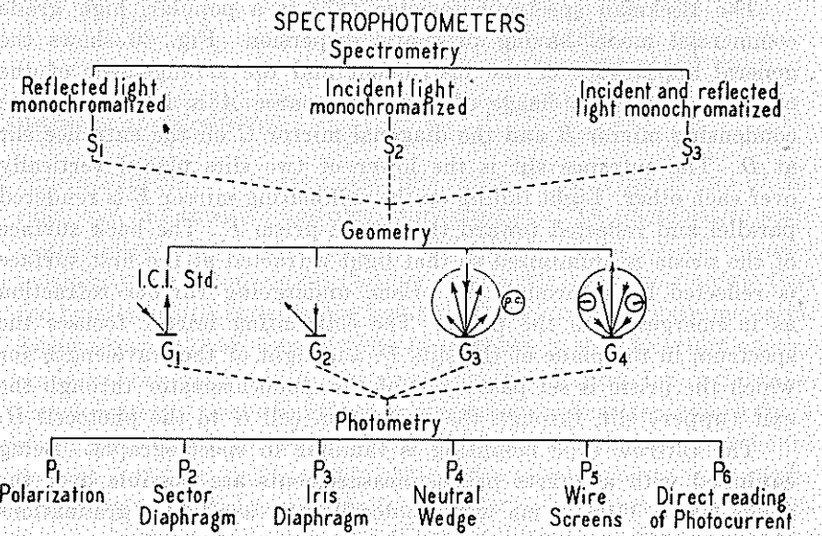



FIG. 29. Diagram showing possible spectrophotometric combinations.

certain kinds of work, especially reflectance of opaque bodies, it is very important to know which kind of instrument is used.* "The most important precaution which is frequently neglected in such measurements is that the spectral reflectance should be measured with optical systems for irradiating the sample and collecting the reflected light in manners spatially equivalent to the conditions of illuminating and viewing for which the color of the object is of interest" *(34d)*.

a.' **Manually Operated Instruments.** For the present purpose, these instruments may be considered as non-recording and recording types. Until recently the former group used only prismatic dispersion in the monochromator. Now several use a grating for dispersion, a construction resulting in simplification and lower cost.

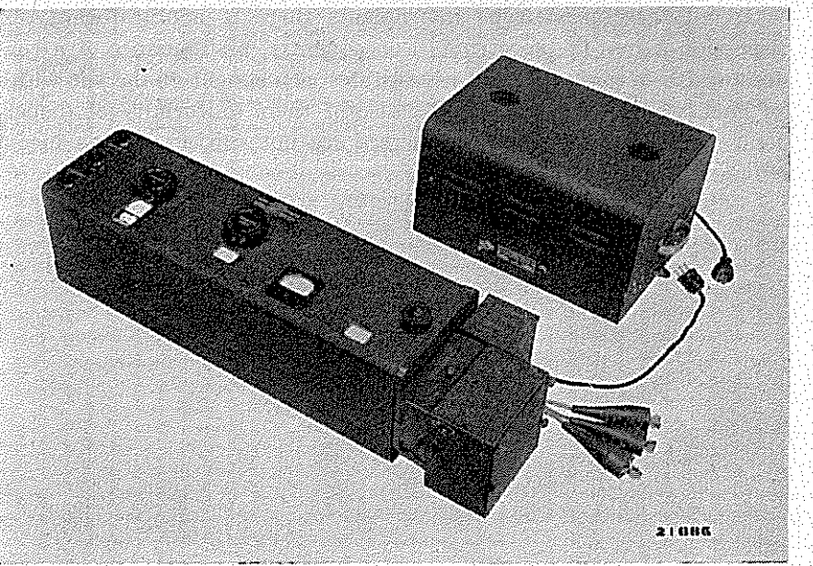
Non-commercial, prismatic instruments, of different degrees of complexity, are represented by those of Barton and Yoe *(14)*, Clark *(30)*, Hogness, Zscheile, and Sidwell *(97)*, and Jacobson, Bent, and Harrison *(103)*. Zscheile's new assembly *(251)* is built around a Hilger-Müller double monochromator. This noteworthy instrument will operate on a spectral band a few Angstroms wide, thus making feasible accurate quantitative determination of mixtures of two such closely related compounds as alpha and beta chlorophyll *(33)*. Kortüm *(116)* has discussed such photoelectric spectrophotometry critically.

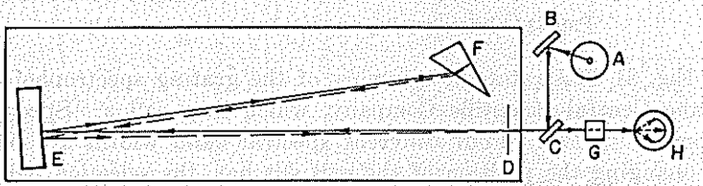
The Beckman spectrophotometer *(27)* is a popular, high grade commercial model having prismatic dispersion. Fig. 30 shows the general appearance of the instrument and the arrangement of the optical system. An image of the light source $A$ is focused by the condensing mirror $B$ and the diagonal mirror $C$ on the entrance slit at $D$. The entrance slit is the lower of two slits placed vertically over each other. Light falling on the collimating mirror $E$ is rendered parallel and reflected toward the quartz prism $F$. The back surface of the prism is aluminized so that light refracted at the first surface is reflected back through the prism, undergoing further refraction as it emerges from the prism. The collimating mirror focuses the spectrum in the plane of the slits $D$, and light of the wavelength for which the prism is set passes out of the monochromator through the exit (upper) slit, through the absorption cell $G$ to the photocell $H$.

The Littrow type mounting is familiar in spectrographs. Being equipped with a quartz prism, measurements are possible over the range 200 to 1100 + m$\mu$ with photocells now available (graduations

* NOTE: No attempt has been made to summarize the extensive literature relating to reflectance measurements (See, however, Ref. *217*).





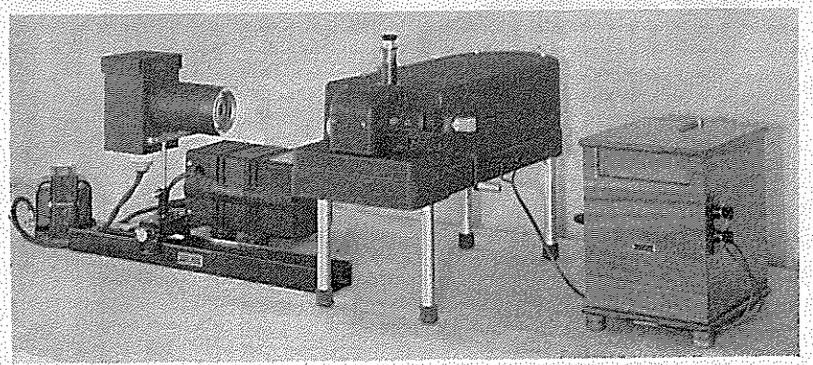



(Courtesy of National Technical Laboratories)

FIG. 30. Beckman spectrophotometer, showing (a) general view and (b) arrangement of optical parts.

run to 2000 m$\mu$). Covering even this range requires use of two photocells, one red-sensitive and one blue-sensitive. The photometric scale is graduated in both transmittance and optical density. High response sensitivity makes operation feasible with a slit width so narrow that the band width is stated to be less than 2 m$\mu$ except in extreme cases. Since adjustment of the slit width is necessary to give a certain energy response, the band width will generally be different for each wavelength setting. Consequently, one can not state that the values for a given curve are all for a given band width. In this case authors should note at least the limits within which the measurements were made. Accessory attachments provide for a variety of measurements, including the continuous recording of the transmittancy of flowing samples of gases or liquids at any one wavelength.

In all these prism-type instruments there is non-uniformity of dispersion across the spectrum. Consequently, maintenance of a constant width of spectral band necessitates adjustment of the slit width for each wavelength setting. Because of their uniformity of dispersion, grating-type spectrophotometers are not subject to this inconvenience regarding band width.



(Courtesy of Central Scientific Co.)

FIG. 31. Cenco grating spectrophotometric assembly.

Fig. 31 illustrates the assembly of the grating spectrophotometer of the Central Scientific Company, which they call a "Spectrophotelometer" *(190)*. Four different spectral band widths are possible by changing the position of the exit slit bar, the selection being 20, 10, 5, and 2.5 m$\mu$.

The Coleman Electric Company has three grating instruments *(32)*. Fig. 32 shows a diagram of the electrical and optical arrangements of Model 10, which contains a double ("DM") monochromator and has a selection of band widths comparable to the "Cenco" instrument. With either of these two instruments the narrowest band width enables one to determine with considerable reliability the transmittance curve for a material such as didymium glass (See Fig. 36). The Model 11 instrument ("Universal"), as illustrated in Fig. 33, operates on a spectral band of some 35 m$\mu$, which of course makes the results less reliable for transmittance curves having small, sharp absorption bands (See Fig. 36). Attachments provide for making fluorescence or nephelometric measurements. A still simpler instrument is the "Junior" Model, advertised especially for clinical determinations.

b.' **Automatic Recording Instruments.** Because of the fatigue accompanying the use of visual instruments, and of the time required



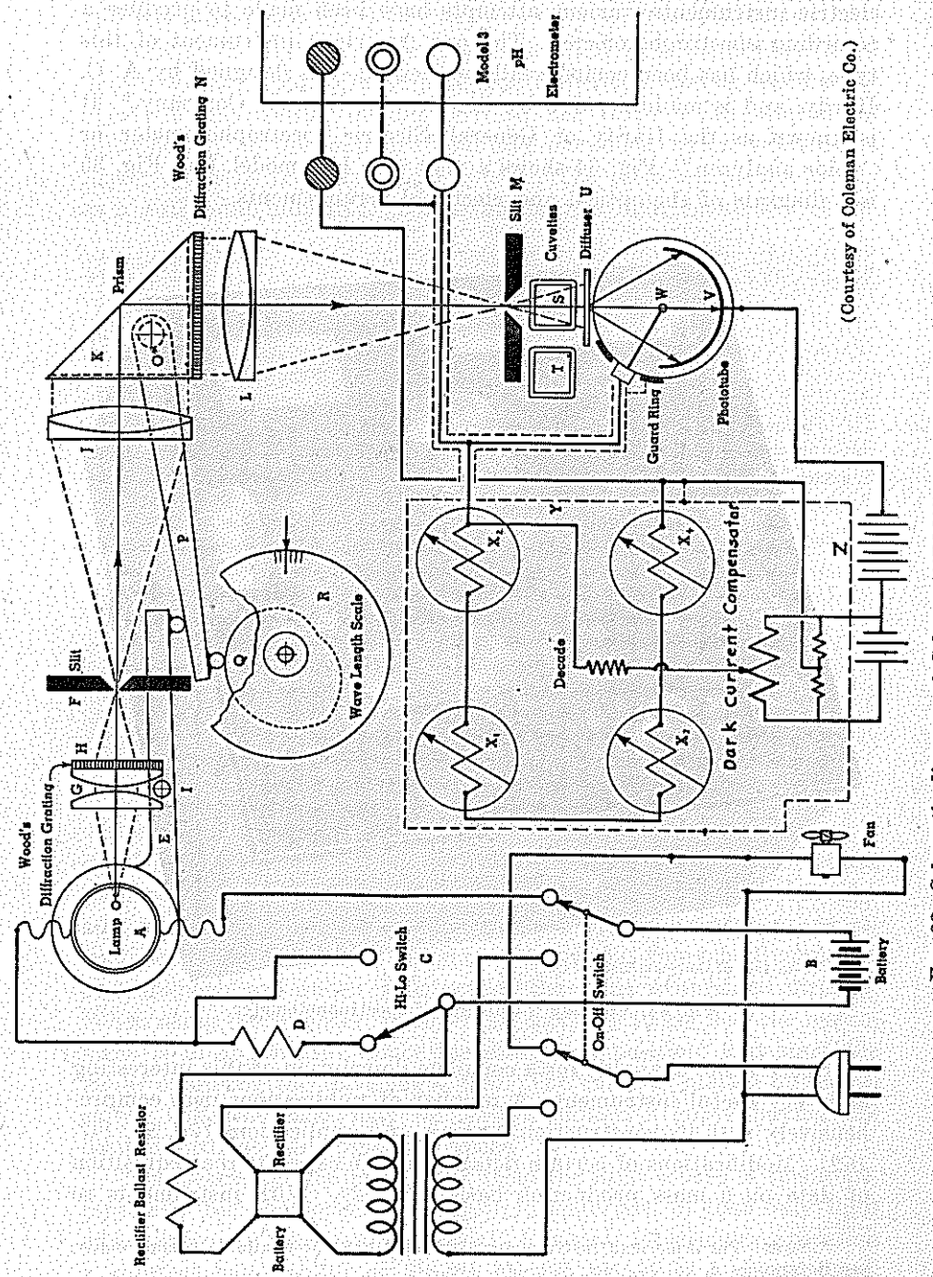



FIG. 32. Schematic diagram of Coleman "DM" spectrophotometer.

to determine a curve with either visual or manually operated photoelectric instruments, various attempts have been made to produce a recording spectrophotometer. The one American instrument of this type, which has been commercially successful, was designed by A. C. Hardy, and is manufactured by the General Electric Company.* It is known as the Hardy or General Electric spectrophotometer or "color analyzer." Fig. 34 shows a view of a late model, and Fig. 35 is a diagram of the optical and electrical arrangements.

(Courtesy of Coleman Electric Co.)

FIG. 33. Coleman "Universal" spectrophotometer.

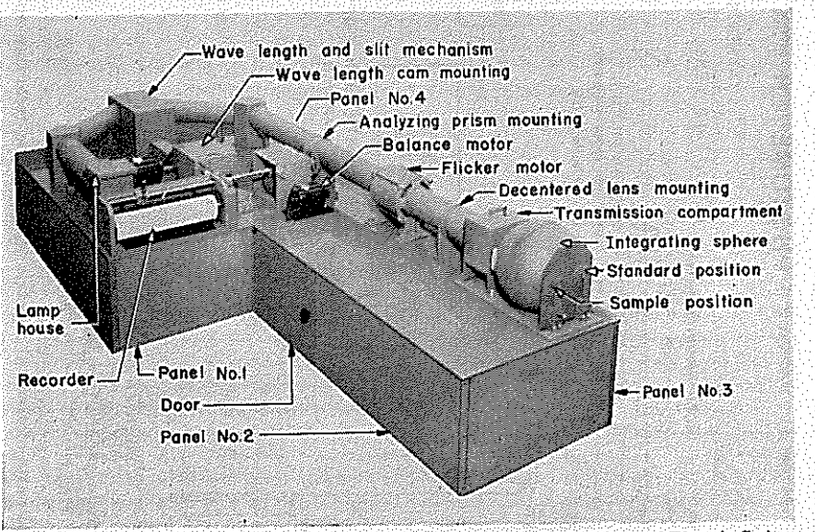
Space is not available for a complete description of this interesting and very useful instrument. A number of publications deal comprehensively with its construction, testing, and applications *(70, 84, 147, 169)*. Modifications of certain details serve to expedite the acquisition of data on a mass production basis *(1, 202)*. With instruments so

* NOTE: For American instruments assembled but not available commercially, see References *2, 25, 76, 153, 170, 178, 198*.



equipped, one concern is said to handle more than 150 samples per day per instrument.

Briefly, the instrument consists essentially of a Van Cittert-type double monochromator, with prismatic dispersion, and an electrically operated polarization photometer. In use there is an alternating illumination of the standard and the unknown, which produces a flicker effect in the integrating sphere. The photocell serves merely as a null point indicator. Whenever the two light beams are of un-




(Courtesy of General Electric Co.)

FIG. 34. General view of General Electric recording spectrophotometer.

equal intensity, the photocell actuates a balance motor which rotates the first rochon prism until equality of illumination is achieved. For any given setting of the wavelength scale the transmittancy (or reflectance) may be read on the photometer scale. Since both standard and unknown are under illumination, the value is obtained directly. When using the recorder, the operator has only to see that the instrument is in adjustment, place the sample in position, and start the motor operating the wavelength cam. The curve is plotted automatically. Different cams may be used to obtain a curve having the desired ordinate and abscissa scales.

The location of the sample far from the light source is fortunate, especially for photochemically-sensitive materials, in that only light of low intensity reaches the material. For most satisfactory operation a spectral band width of 10 m$\mu$ should be used. A 5 m$\mu$ band,

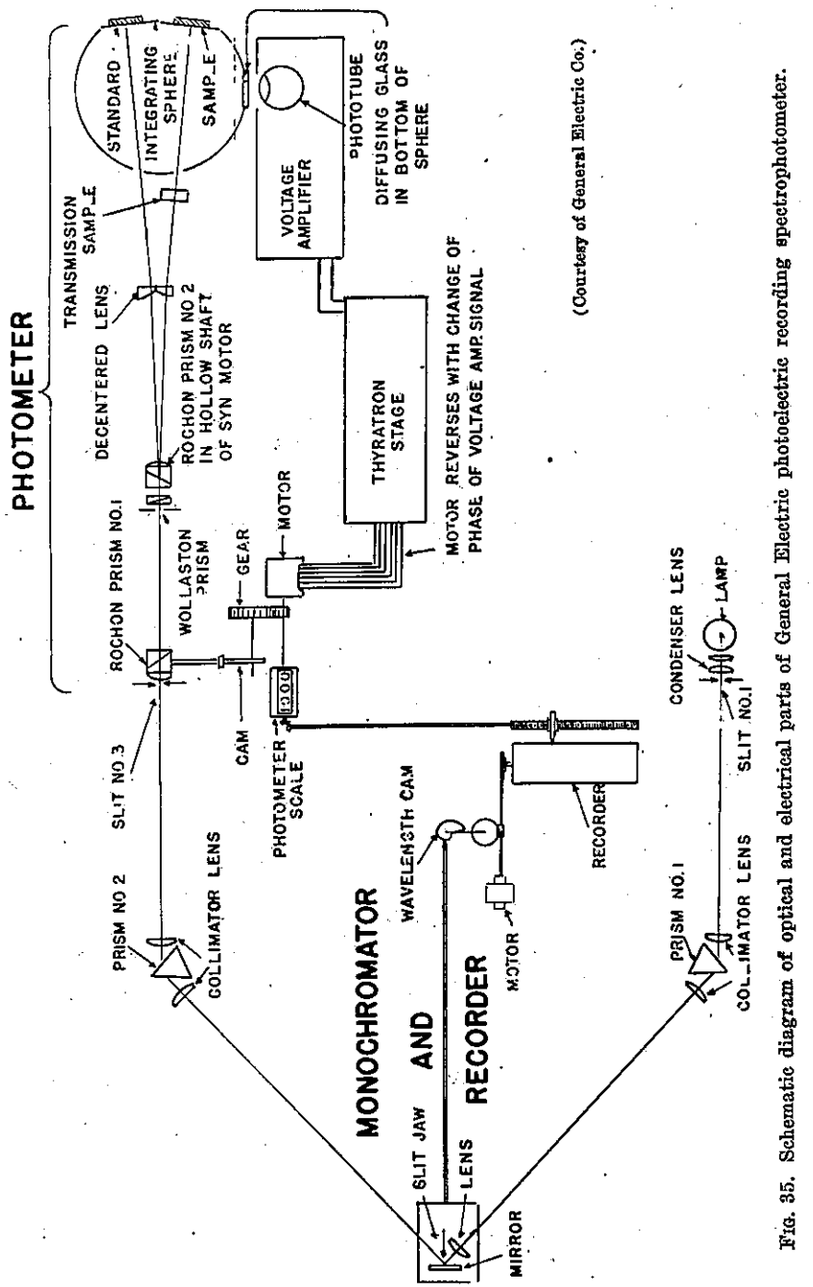



Fig. 35. Schematic diagram of optical and electrical parts of General Electric photoelectric recording spectrophotometer.

possible with carefully controlled adjustments, is best for curves of the kind shown in Fig. 44. For relatively non-selective curves 3 to 5 minutes per sample are required, but more time is necessary for steep curves and narrow band widths. The instrument is so complicated and the sources of difficulties are sufficiently varied and frequent that only a skilled operator may be expected to obtain reliable data with it. Printed statements that a physicist is necessary as an operator are in error.

A more recent instrument, devised by Harrison *(86)*, uses a grating in the monochromator. Its range is from 200 to 1000 mμ in contrast to 400 to 750 mμ for the Hardy instrument.

**Nature of Spectrophotometric Data.** Lack of accuracy, clarity, and consistency characterizes the current presentation of too many absorptive spectrometric data by chemists. Some of the significant items which should be considered follow *(139)*.

a. **Instrumental Details.** Turning to the evaluation of the data, one is interested first of all in their reliability. Since different instruments, and different methods of using them, may yield different results, reports of the details of measurement must be clearly and accurately written. It is analytically inadequate, for example, to state only that the results were obtained by some individual, or that they came from some individual's laboratory.

In the first place, one wants to know the type of instrument—that is, whether it was photographic, visual, or photoelectric. Each type includes instruments of different qualities. If a photographic instrument was used, one may want to know whether any point matching of lines was done visually or by photoelectric means. An estimate of the probable reliability of the readings would help. Perhaps most important is to know whether a visual type was used, and, if so, with what light source. In addition to stating the type of instrument, authors should report the manufacturer and model. Thus, Coleman Model 10 S will operate on a spectral band as narrow as 2.5 mμ, but that used with Model 11 is 35 mμ.

Current papers seldom mention calibration. It seems necessary to check rather often both wavelength and photometric scales. Monochromatic sources, such as lines of the mercury arc, are preferable for checking wave lengths. Band peaks of didymium glass (See Fig. 36) serve for rough checking of recording instruments if one uses the slit width employed in calibrating the glass. Carefully selected glasses, calibrated by the National Bureau of Standards, are very useful for checking photometric values. Several solutions have been suggested for this purpose *(36, 195, 222)*.

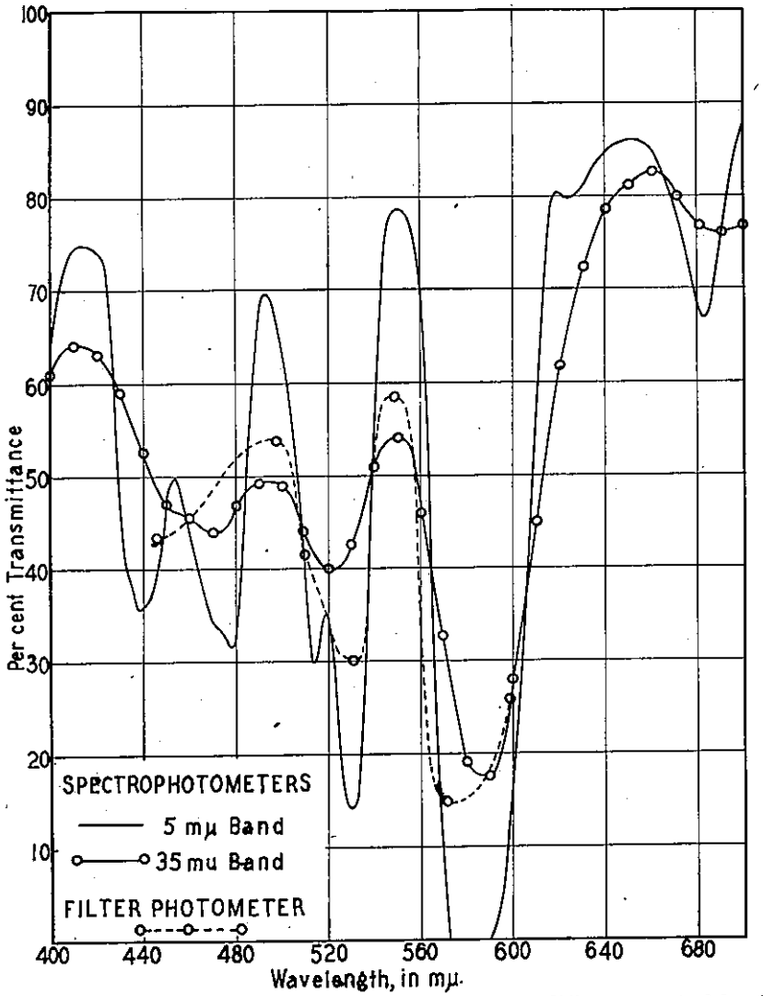



FIG. 36. Transmittance curves for didymium glass using different spectral bands.

For curves having small, sharp bands the significance of spectral band width seems often not appreciated or understood. Many authors do not state the width used. Fig. 36 shows the results of using different band widths for a didymium glass. The continuous curve, without points, was recorded with a band of 5 mμ, while that with the marked points is based on readings with a band of 35 mμ. The discontinuous curve is drawn through points marking the values obtained on a visual photometer using glass filters whose median wavelengths were stated by the manufacturer to be the values locating



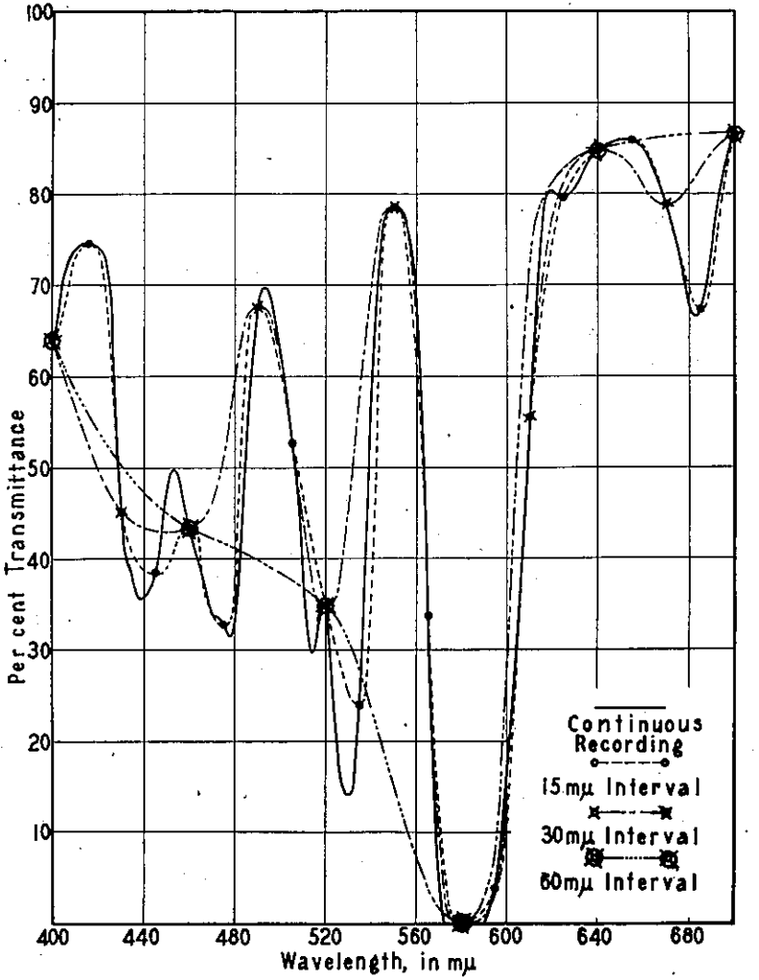



FIG. 37. Transmittance curves for didymium glass using readings taken at different wavelength intervals.

the points on the graph. The disagreement is obvious. In general, the use of a wide spectral region obscures or eliminates small bands which might well be the characteristic information sought. Two recent publications, from well known laboratories, contain curves with no sign of the small bands known to be in the permanganate curve near 490, 508, 526, 545, and 567 mμ *(81)* (See Fig. 38).

If narrow spectral band widths were used for these permanganate curves, perhaps too few points were taken to locate the curve. Unless

data come from a recording instrument, the observed points should appear in the graph. Except for curves relatively non-selective, photometric readings should be taken at least each 10 mμ. To bring out small absorption bands, the points must be closer. Fig. 37 shows variation in the curves obtained by plotting the readings taken at different wavelength intervals for the glass used in Fig. 36. It is evident that too few values can lead to serious inaccuracy for such a curve.

Along with pointless curves, one sees too many gridless graphs. The grid lines, because of their aid in studying and using curves, are recommended in books dealing with graphical presentation of data *(10, 238)*.

If one wants to repeat another's work, it is very disconcerting to find no mention of the concentration of a solution or the thickness of sample measured. For the sake of easy comparison of data, generally absorption cells 1.00 cm. thick, or some multiple or fraction thereof, are preferable. But if the actual thickness, whatever it is, is given, Bouger's law enables one to calculate to any other thickness. Just as with wavelength and photometric scales, absorption cells should be checked for internal thickness.

**Data and Conventions.** If one has an instrument capable of yielding results of the desired accuracy, and if it has been adjusted and calibrated, there remains the question of how to handle the experimental data obtained.

Although the terms absorptometric and absorptance are used, more often the measurements are reflectance, $R$, for an opaque material, and transmittance, $T$, (or transmittancy, $T$), for a transparent system. Reflectance is generally expressed as percentage. This basis is often used also for transmittance of solids and transmittancy of solutions. Hardy *(83)* and others prefer the decimal fraction, transmittance (or transmittancy) factor. Also there is extensive use of optical density, $D$ (designated by many as extinction, $E$), the specific extinction coefficient, $k$, the molecular extinction coefficient, $K$, or the logarithm of one of these. A number of instruments read directly in terms of one or more such values. Occasionally the graduation of the photometer is in terms of a 90° sector angle or of a 45° polarization angle. Brode *(22)* gives tables for the interconversion of such values. Whatever the basis of the readings, they form the ordinates for curves. The region of the spectrum measured forms the abscissas, and the values are expressed in one or more of the following terms: wavelength, λ (in millimicrons, mμ, or Angstroms, $A$), frequency $\nu$ (in fresnels), wave number, $\nu'$ (in waves/cm.), and logarithm of the



wavelength. Wavelength, wave number, and frequency are related according to the expression

$$\frac{1}{\text{Wavelength}} = \text{wave number} = \frac{\text{frequency}}{\text{speed of light}}$$

wavelength being mμ × $10^{-7}$ cm., and speed of light being 3 × $10^{10}$ cm./sec. Brode also gives tables for interconversion of these values. From the standpoint of the accuracy of the measurement, few graphs would seem to justify the significant figures implied in using Angstrom units.

Unfortunately, no one system of plotting has been generally adopted. Thus, a recent publication uses six different ordinate designations. This situation is the result partly of personal inertia and prejudice, and partly of the inadequacy of any one system for all requirements. With all possible variations in use, including inconsistency in the direction of plotting for any given combination, and disregard for good practice in graphing, the literature is likely to be confusing to one unskilled in transforming mentally a curve in unfamiliar form over into the form with which he customarily deals. Some advantages of several forms may be noted.

Transmittance (or transmittancy) is often the quantity obtained directly, especially with single-beam, substitution or recording types of photoelectric instruments. Thus, presentation of the original data becomes easy. Since the light transmitted (reflected) determines the color, this kind of plotting is preferred by many in the designation of colors. Also, curves in this form are the basis for calculating numerical color specifications, as noted later. Some writers plot log $T$ on equal-division paper, or $T$ directly on semilogarithmic paper. Either method greatly magnifies values below 10 per cent, in comparison to those above 80 per cent.

Optical density (extinction to many) magnifies absorptance maxima, and thus makes them definite and easily read. Also it facilitates computation from one thickness to another, if desired, since the relation is linear.

Curves of the percentage-wavelength, or optical density-wavelength type, often differ considerably in shape for different thicknesses of media or different concentrations of solutions. If one plots log $D$ (or $E$), the curves all have the same shape *(83)* regardless of thickness (or concentration, if the solution conforms to Beer's law). This is evident from the relation,

$$D = \log_{10} 1/T = 0.4343\, kx$$

Then

$$\log_{10} D = \log_{10} 0.4343\, k + \log_{10} x$$

Since the coefficient $k$ varies with the wavelength, and since the thickness (or concentration) of the sample, $x$, does not, the shape of the curve depends upon the term $\log_{10} 0.4343\ k$, and the height upon the term $\log_{10} x$. The optical density, $D$, may be plotted, of course, on a log scale paper.

In some laboratories this type of curve is used for standard curves and for the identification of materials. Curves of identical materials are superposable. In dyestuff manufacture and application, for example, the trade thinks in terms of shade and strength, variables immediately separable by this method as curve shape and position variations, respectively. Shurcliff *(193)* has proposed a curve-shape index for identifying dyes by means of spectrophotometric curves. For reflectance data the ordinate is log $(1-R)^2/R$, in which $R$ is the body reflectance *(1)*.

In theoretical and interpretative studies on the relation of absorption and constitution, often including the ultraviolet and infrared regions, frequency is of more fundamental importance than wavelength as abscissas, since it gives a better indication of the relative width of bands in the three regions. Shurcliff *(194)* prefers to use the logarithm of the wavelength (to the base 2), as shown in Fig. 43.

As another point concerning data, mention may be made of uncertainty in definition, and inconsistency in use, of the terms employed. Thus, unless one knows the solutions measured, he can not be sure that a curve labeled absorptance is not really a transmittance curve. Does a writer mean transmittance or transmittancy? When extinction, $E$, is used, does he mean optical density, or specific or molecular extinction coefficient? In any case, has he specified concentration, thickness, spectral band width, solvent, and the wavelength of the measurement?

Finally, note may be made of what seems unjustified faith in published values of optical density or extinction coefficients, including their use for plotting curves intended either for measuring amounts of constituents or for demonstrating the applicability of Beer's law to the system. Ideally, the absorptance should be a physical constant comparable in reliability to other constants, such as refractive index. If the value is determined accurately, under carefully specified conditions, it may be so considered. But when one recalls that Beer's law presupposes monochromatic light, and that few spectrophotometers, as generally used, come near meeting this requirement, the discrepancies reported are understandable. Often a point of doubt is the purity of the material measured. Perhaps this too implicit faith in



extinction coefficients accounts for many authors insisting on publishing extinction coefficient-concentration graphs for their particular work. If the line is straight, a sentence will so state. An experienced worker would not use the curve for another instrument without checking. An illustration of the significance of this instrumental factor has been published by Withrow, Shrewsbury, and Kraybill *(234)*. Sandell *(182)* has wisely cautioned, "Only the most sanguine user of a spectrophotometer will calculate the concentration of his colored solution from the observed extinction and the value of the extinction taken from the literature." The dependence of such values upon conditions is shown by a statement such as

$$D\left(\begin{matrix}1\% \\ 1\text{ cm.}\end{matrix}, 425\ \text{m}\mu, \text{CHCl}_3, 5\ \text{m}\mu, 20°\text{C.}\right) = 1800.$$

Although a single determination or value, at some given wavelength may suffice for a quantitative analysis, generally at least a portion of the visible region of the spectrum is covered for either qualitative or quantitative determinations. The data are presented then in the form of tables or graphs showing the determined values of transmittance or reflectance. Figs. 38 to 43 show curves plotted in various ways for aqueous solutions of potassium permanganate. The curves in Fig. 38 came directly from a recording spectrophotometer, and the others are based on these originals.* It should be noted that the transmittancy curves of Fig. 38 were obtained with solvent in one light beam and solution in the other, using matched absorption cells. Occasionally one sees a graph showing separately the transmittances of the solvent and of the solution (See Ref. *125*).

## APPLICATIONS

In general, the applications of light absorption spectrometry depend ultimately upon measuring color, a systemic property *(145)* of increasing importance in research and industry. Many chemical materials possess characteristic colors, or are subject to color changes

* NOTE: In plotting the data in Fig. 39 it was assumed that $1 - T = A$ (absorptancy). The danger of generalizing such an assumption is indicated in the quotation from the report of the Colorimetry Committee (*54b*). "Computation of absorptance as unity minus the sum of reflectance and transmittance is incorrect in many cases because this quantity includes losses by internal reflection of light scattered in the medium, much of which is lost or absorbed at the edges of the object. Similarly, it is usually incorrect to consider the absorptance of an object observed by transmission as the difference between unity and the transmittance. This quantity includes the losses by reflection as well as the losses within the object resulting from both absorption and scattering. In the case of objects observed by reflection, the absorptance can not be assumed to equal the reflectance subtracted from unity, for this difference includes the transmitted energy as well as the energy that is internally scattered and absorbed."

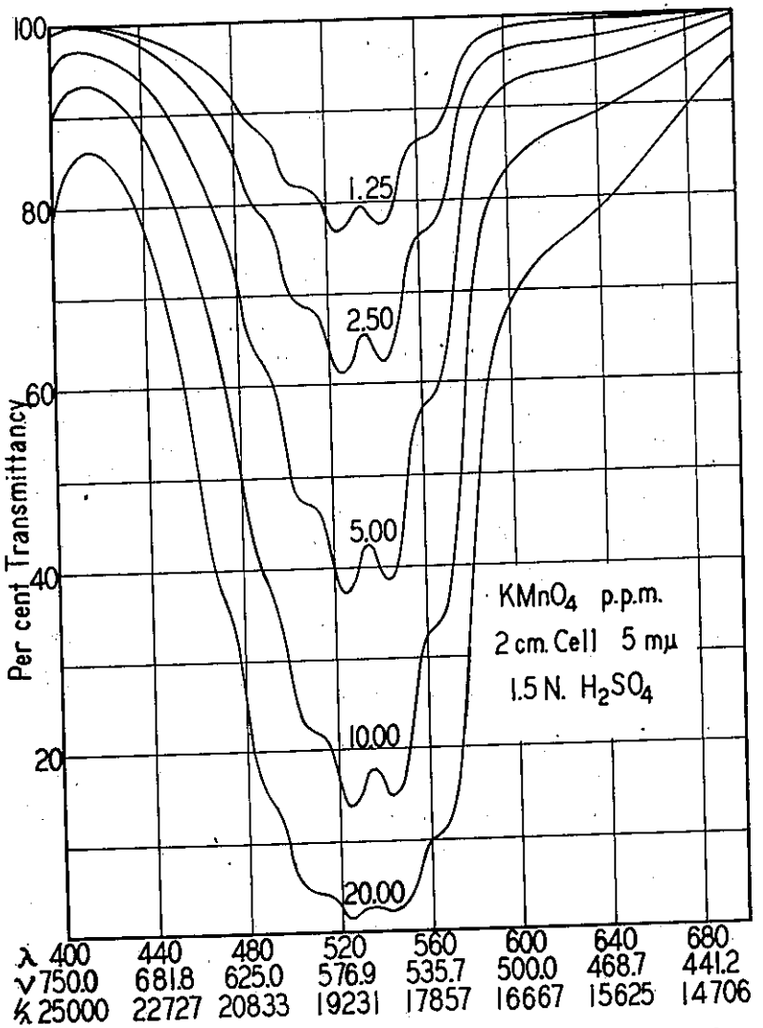



FIG. 38. Transmittancy-wavelength, -frequency, -wave number curves for solutions of potassium permanganate (linear plotting).



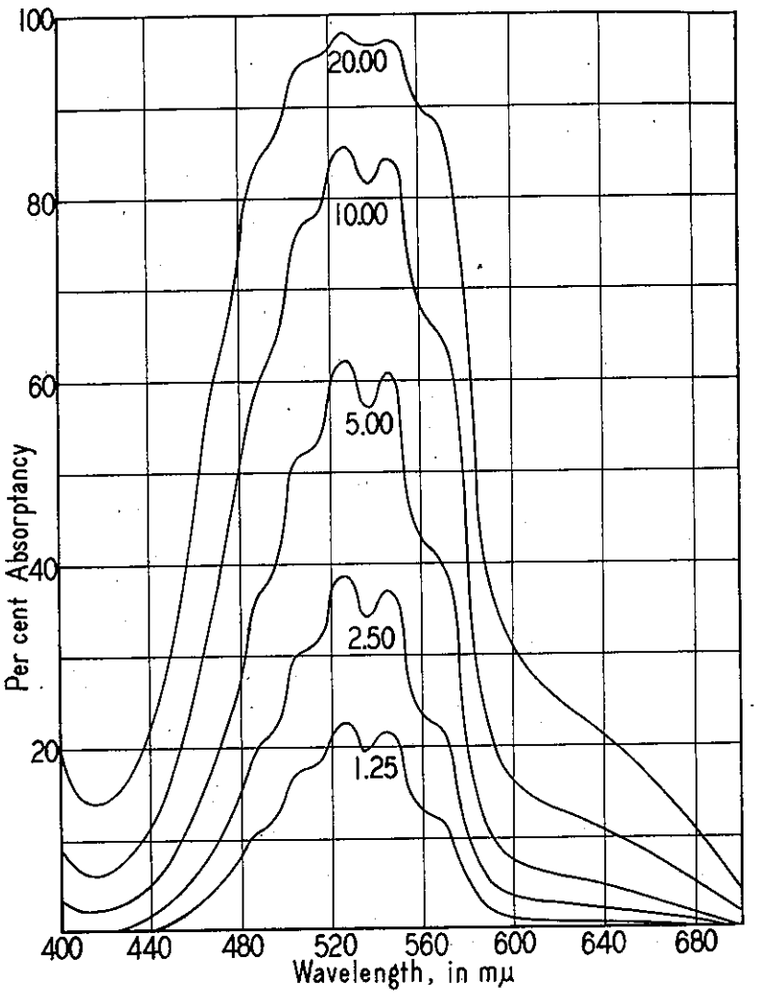



FIG. 39. Absorptancy-wavelength curves for solutions of potassium permanganate.

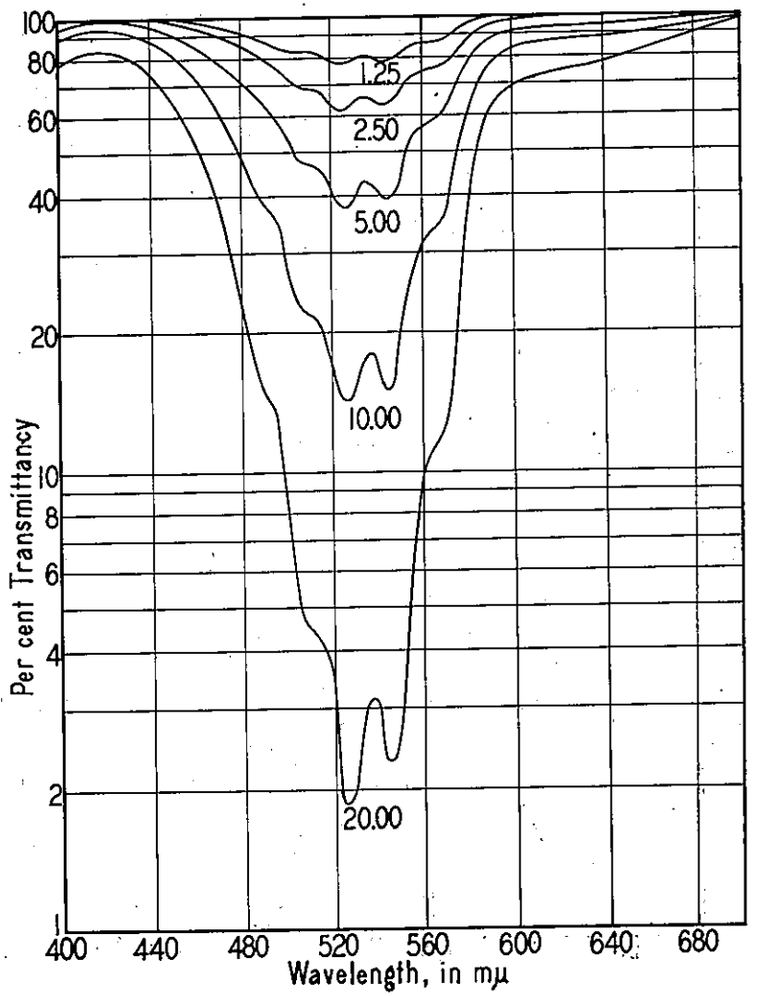



FIG. 40. Transmittancy-wavelength curves for solutions of potassium permanganate (semi-logarithmic plotting).



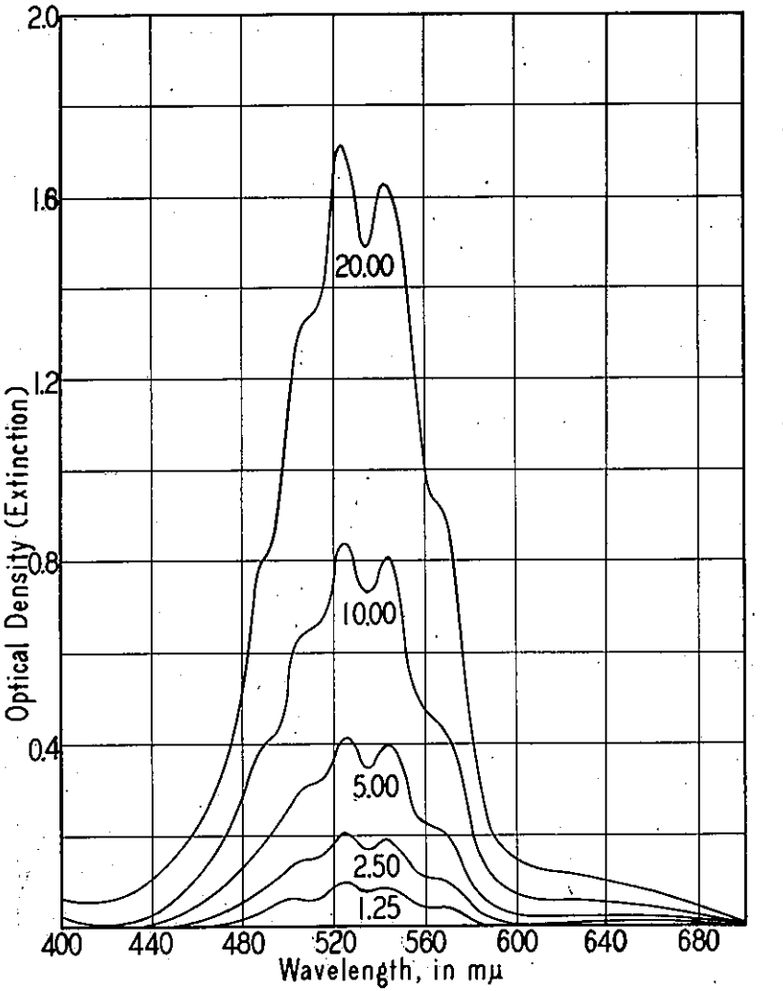



FIG. 41. Optical density-wavelength curves for solutions of potassium permanganate (linear plotting).

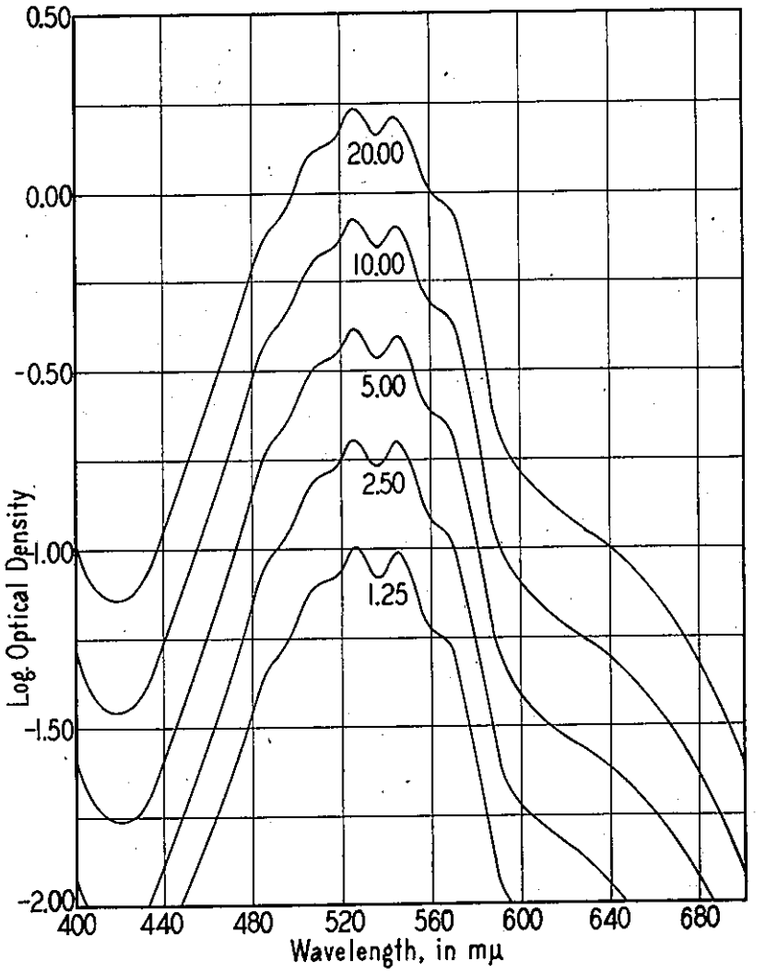



FIG. 42. Log optical density-wavelength curves for solutions of potassium permanganate (linear plotting).



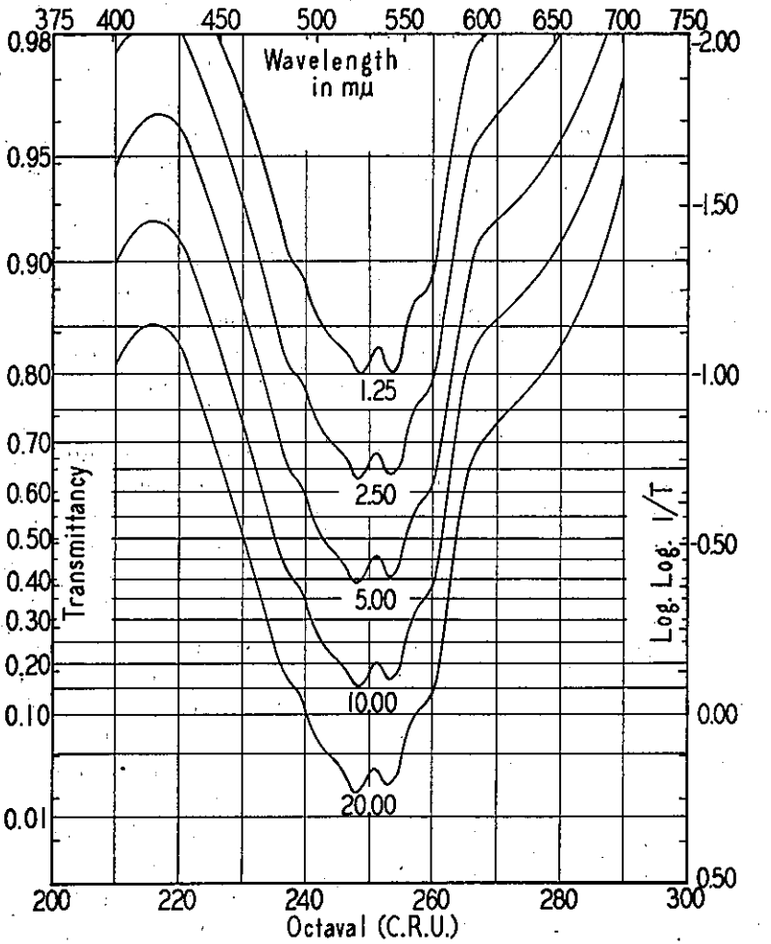



FIG. 43. Log log 1/T-log wavelength curves for solutions of potassium permanganate (linear plotting).

in the course of chemical transformations. The range of application extends from the determination of color as color, through the mass-production determination of desired constituents in routine laboratories, to the elucidation of chemical problems, such as those involving the constitution of systems, or the nature of processes *(21, 139, 140, 149, 154, 202)*.

The objective of the measurement differs widely. Perhaps most frequently it is purely analytical; that is, we want to know what constituent is present and/or its amount. Such determinations may be necessary in industrial control operations, or they may be merely incidental, though necessary, for solving a research problem. In color specification work we probably do not determine what is present, or its amount, although the former largely determines the dominant wavelength and the latter determines the luminance and purity (colorimetric), for a given illuminant. Even though some work is not primarily analytical in objective, it is convenient to consider spectrometric measurements in terms of qualitative and quantitative uses of the data *(144)*.

1. **Qualitative Uses.** The applications which are qualitative in nature depend upon an object's having a definite form of spectral curve (resulting from absorption, transmission, or reflection), with its distinctive parts, if any, in particular regions. Upon these characteristics are based any conclusions deduced from the curves. Some examples will illustrate the possibilities.

a. **Identification of Constituents.** For many substances the most characteristic portion of absorptive spectrometric curves lies in the ultraviolet and/or infrared regions. However, for many other systems the curves for the visible region do have considerable analytical value. As already mentioned, the curve shape may be one of the items used to characterize dyes *(98, 186)*. An experienced observer soon learns the peculiarities of a given system, such as pigments in printing inks *(1)* or a permanganate solution. The whole range of purples, from bluish to reddish, may be interpreted in terms of the respective curves. Even with systems such as dark blues and browns, whose curves appear quite uninteresting, one learns what to expect from given constituents. As an example of unusually interesting absorption in the visible region, the curves of Fig. 44 are included.

In some work, where the curves do not have sufficiently sharp absorptance maxima for such identification, it has been found preferable to use the ratio of extinction coefficients at selected wavelengths *(99)*.



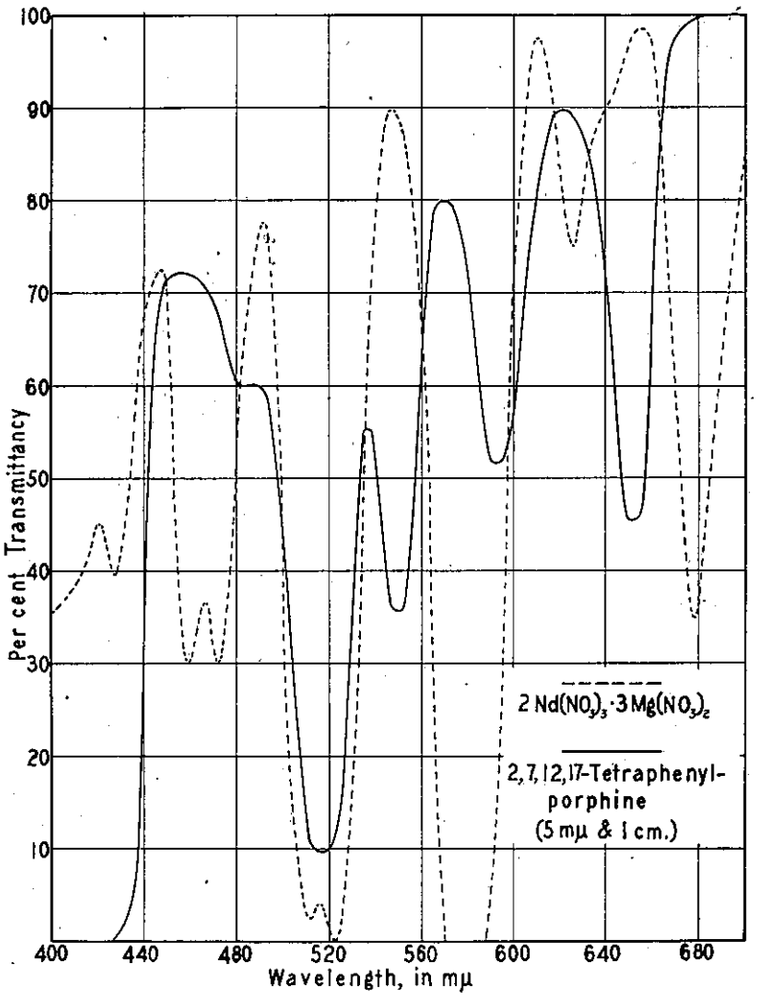



FIG. 44. Transmittancy curves showing characteristic absorption bands in the visible region.

This general use of curves has been very extensive in organic chemistry in studies involving correlation of absorption spectra and constitution of compounds *(20, 21, 26, 71, 73, 89, 96, 149, 154)*. If the curves for an unknown and for a known agree closely, identity of composition or structure is indicated, but not confirmed, as noted by Ruehle *(180)*, for two systems containing quite different substances may yield closely agreeing curves *(205)*.

Spectrophotometric evidence on the nature and the course of chemical reactions belongs to this category. Such applications, although probably most extensive in biochemistry and organic chemistry, is not confined to these subjects *(155)*. Specific examples of these applications are papers on adsorption *(131)*, complexation *(151)*, hydrolysis *(28)*, ionic equilibria *(17, 75, 176, 224)*, ionization constants *(181)*, isomerism *(173)*, molecular association *(119)*, polymerization *(175)*, and solvation *(228)*. To interpret curves one should know the light absorptive effects of factors such as functional groups, specific structures, and possible chemical transformations *(96)*. Change in hue, for a given illuminant, means a change in the nature of the absorbing system, although the reverse is not necessarily true. Theoretical aspects of the interpretation of absorption spectra have been presented by many including Lewis and Calvin *(126)*.

b. **Study of Colorimetric Standards.** In certain analytical methods, such as the comparimetric determination of residual chlorine in water, the nature of the colored system is such that matching with a similarly prepared known solution is impractical. So-called permanent standards, composed of other substances, usually glass disks or aqueous solutions, are used. They are designed to be visually equivalent to the unknown. If spectrophotometrically equivalent (have the same curves), they will match under any kind of illumination. If not, one could have difficulty. Two systems may appear visually identical, under given illumination, and yet show quite different curves *(137)*. Fig. 4 shows the curves for three "matching" solutions. A study of such systems led to the interpretation of certain difficulties with the o-tolidine method for chlorine *(44)*. Incidentally, it is of interest to note that the chlorine-o-tolidine yellow solutions have a rather sharp band at 436 mμ, but the best visual matching solutions do not. Nevertheless this difference in absorptive capacity of the two systems in the blue is not serious for matching, presumably because of the low sensitivity of the eye in this region. Systems exhibiting dichroism *(241)* may give trouble in colorimetric matching.



c. **Selection of Filters.** A daily problem for many analysts is the selection of filters for the filter photometers which now find extensive use in the colorimetric determination of constituents in a wide variety of materials in many industrial and clinical laboratories. The best basis for such selection is the spectral transmittance curves of the system to be measured and of the available filters. To achieve maximum sensitivity in the photometer, the usual advice is to use a filter whose maximum transmittance is close to the wavelength of the peak of the absorption band of the unknown. Fig. 17 shows curves for the 1,10-phenanthroline-iron complex and for two glass filters. The wide line centering near 508 mμ is drawn to scale to indicate the setting of a system to pass a spectral band having 5 mμ width at the peak of the absorption band.

Occasionally, if the curves for filters and constituents are suitably related, it is possible to work with more than one colored substance in solution. An example has been discussed by Knudson, Meloche, and Juday *(114)*. Usually colored color-forming reagents are undesirable because any excess added increases the total color, as in the determination of iron with nitroso-R-salt. Inspection of the curves for this reagent and for the iron complex shows the possibility of filtering out the former's color.

d. **Control of Variable Factors.** Probably few chemists appreciate the importance of color in connection with chemical products and processes. Surprisingly often the production, or avoidance, of given products, or the control of production and operational processes, is based upon color phenomena. In analytical chemistry especially, one or more of at least the following factors are often very important: stability of the color, pH change, temperature, time of reaction, best color-forming reagent, amount of reactant required, order of mixing reactants, state of oxidation, nature of the solvent, and interfering ions. Studies of this kind in the author's laboratory *(57, 113, 156, 157, 206, 236, 239)* aimed to determine the factors affecting various colors and their formation, and then to establish working conditions that would yield acceptable analytical results.

In terms of the ultimate effects upon the spectral transmittance curves, two kinds of results may be recognized. One is a change in hue, which is indicated by a horizontal shift of a curve or by a change in its form (to avoid concentration form-change, one may plot log $D$ as ordinates). Such shifts of position, or real change in the form of the band, means a change in the nature of the composition of absorbing medium, which is the solute for a given solvent system. It may lead to identification of constituents, as already mentioned.

Diverse, colored ions generally change a hue, as do acidity changes in systems subject to pH action. Occasionally a system, such as aqueous cobaltous chloride, is rather sensitive to changes in temperature or to the nature of the solvent.

The second kind of result is a change in intensity, or luminance, accompanied, of course, by a change in purity (colorimetric). This is shown by a change in the height of the peak of the absorption band. Any action inhibiting the absorption process is made evident by fading and accompanies a decrease in the absorption band. Examples are diverse ions reacting with the color forming reactant to give a colorless component or to complex the desired constituent to prevent its functioning. Increase in the band means enhancement of the absorptive capacity by some reaction that must be controlled or prevented.

All these cases necessitate the establishment of some set of conditions to obtain standard reference or working curves. Assuming the applicability of Beer's law, we have a basis for measuring the magnitude of the intensity effects in terms of the desired constituent *(57, 206)*. If the effect so calculated does not exceed two per cent, it is generally disregarded in the author's laboratory, because so much colorimetric work is not within this limit of accuracy.

Finally, for a variety of materials there may be established, in terms of curves, standard specifications of quality and performance. Being permanent, these curves serve for the comparison of subsequent products. In a paint or dye laboratory, for example, data are secured for the standard or desired grades of materials. Then the effects of processing, impurities, and other measurable factors can be determined in terms of the standard curves. This may lead to change and control of the manufacturing process to achieve permissible color tolerance in the products. Two examples of such uses are for paint *(60, 100)*, and for dyes and textiles *(35, 45, 152, 163, 165, 191)*.

e. **Provision of General Information.** For many systems analysts are interested in the curves in a more or less general way. The information may lead to general conclusions about the nature of the system, or it may serve for the adoption of specific details in a given situation.

Two studies of acid-base indicators may be cited as examples of work undertaken to satisfy a curiosity concerning the colorimetric characteristics of certain systems. Fortune *(56)* investigated a number of simple, modified, and mixed neutralization indicators through



the pH range of their hue transformations. Most interesting was the effect of the hue modifier in the modified indicators, especially in the production of a nearly colorless intermediate stage. Subsequently, Woods *(237)* extended this work to include the so-called universal indicators in order to determine the nature of those proposed and to establish, if possible, a basis for their improvement. Brode has summarized other examples *(21)*.

2. **Quantitative Uses.** Quantitative uses of light absorption spectrometry depend upon the fact that the magnitude of absorptance is a function of the concentration of the absorber, which in liquid systems is the solute. The measurements provide the basis for securing a variety of quantitative information *(12, 218, 242)*, representative types of which are summarized here.

In the analytical determination of constituents it is customary to make the measurements at the wavelength of the peak of the absorption band, although this point is not always the best. With unstable systems the optimum spectral zone may not be that of maximum absorptance *(204)*. Also, with more than one colored constituent present, it may be necessary to measure one component on a steep portion of a curve to avoid interference by the second component *(192)*. Finally, when simultaneous equations are involved in the calculation, readings are necessary at several points *(33)*.

a. **Sensitivity and Range of Method.** A set of curves for a series of solutions of suitable concentrations shows, for the specified thickness, the sensitivity of a method of analysis and the range of concentration to which it may be applied reliably without changing the conditions, such as diluting or concentrating the sample or using different cell thicknesses. If desired, data for one thickness may be calculated to those for another on the basis of Bouguer's law.

The curves for Fig. 17 show the possibilities for a better than average colorimetric method. Some methods are more sensitive, but most of them are not the equal of this one. Fig. 38 shows a similar set of curves for the determination of manganese as permanganate.

As pointed out already, such curves are used for the selection of filters, and, more important, they are fundamental for the calculation of I.C.I. numerical specifications (See discussion of color analysis).

b. **Conformity of Solutions to Beer's Law.** A set of curves such as those described in 2a, will serve to test a given system for conformity to Beer's law. First the transmittancies or the optical densities for the various concentrations are noted at the wavelength of maximum absorptance, or as near this point as feasible, if it lies outside the

visible range. Then one usually plots as abscissas the concentrations, and as ordinates the optical density or the logarithm of the transmittancy, on a linear scale, or the transmittancy on a logarithmic scale. Straight lines show conformity, a fact which ordinarily needs merely to be stated in a paper. If such a curve is to be used for calculations based on the additive nature of optical densities *(33)*, the data should be determined for the instrument used. Fig. 45 shows Beer's law curves for the permanganate curves of Fig. 38. It will be noted that transmittancy readings were taken for three wavelengths, 525, 500, and 475 m$\mu$, the first being that of maximum absorptance. The straight lines show conformity at all three wavelengths, but the slope of the lines is best for the readings at 525 m$\mu$.

Non-conformity to Beer's law indicates the desirability of making comparimetric measurements with a constant depth standard series method, rather than with a variable depth instrument. Such deviation may be valuable qualitative evidence of some action affecting the absorber, such as association, dissociation, or ionization.

c. **Analytical Determinations.** The possibility of applying spectrophotometry for making determinations of constituents by means of absorption has been recognized since a publication appeared by Vierordt in 1873 *(221)*. For some years such methods have been appreciated by physicists. More recently chemists have begun to apply the procedure to a variety of systems. Representative examples include dyes *(8, 9, 23, 45, 67, 99)*, chlorophyll *(248)*, hemoglobin *(37)*, hydrogen ion concentration *(24)*, nitrogen *(129)*, vitamins and hormones *(154)*, and general applications *(214)*.

As reliable apparatus has been expensive until recently for the ordinary laboratory not specializing in such work, this kind of method has been of value chiefly in situations where some other adequate procedure is not available, as with Zscheile's work on chlorophyll *(248)*. Now the relatively inexpensive small grating instruments will undoubtedly extend such applications.

a.' **One Constituent.** A common method for determining one constituent was applied by Mehlig to manganese in steel *(134)*. The procedure depends upon the fact that the transmittancy at a given wavelength is a function of the concentration for a solution such as permanganate. If a reference curve is constructed, plotting the transmittancy, at a given wavelength, of a series of standard solutions of permanganate against the known concentration of manganese, it is possible to convert the transmittancy of an unknown permanganate solution to concentration of manganese by use of the curve. The



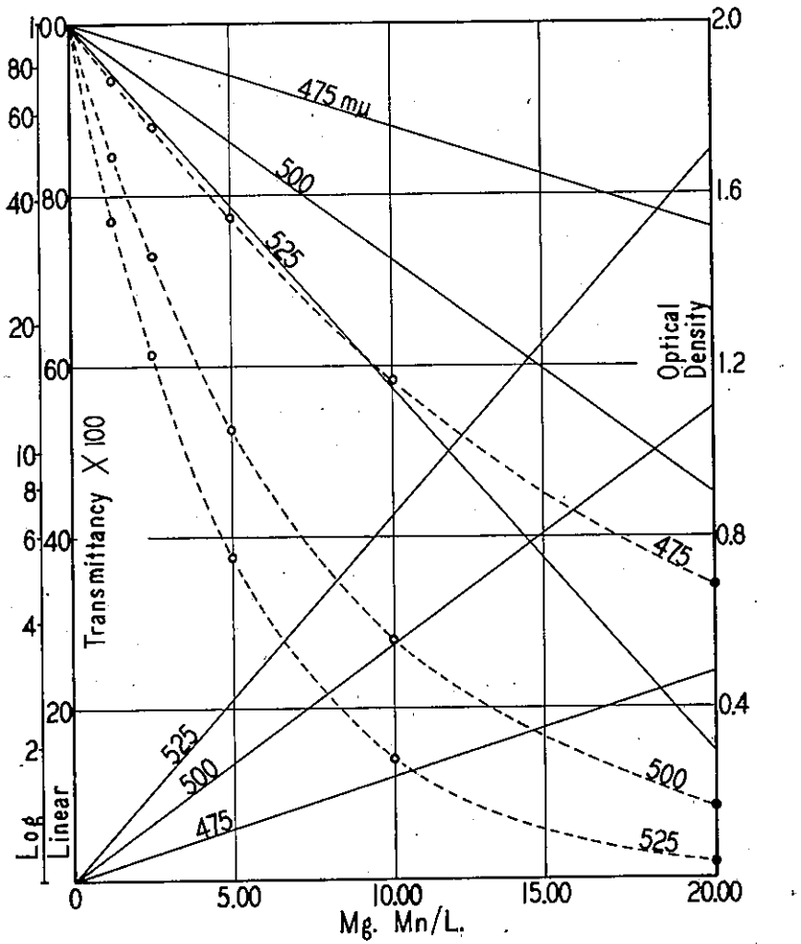



FIG. 45. Transmittancy and Beer's law curves for solutions of potassium permanganate at three wavelengths.

broken lines in Fig. 45 show such curves for three different wavelengths taken from the curves of Fig. 38.

Before constructing such a reference curve, one should determine the transmittancy curve from 400 to 700 mμ for the kind of solution to be measured in order to be able to select a suitable region for making the readings. The preferable wavelength is where there is least change in transmittancy for a given change in wavelength; that is, where there is a minimum (or, rarely, a maximum) in the transmittancy curve. On steep portions of a curve a small error in wavelength results in too great an error in the transmittancy. For visual instruments the wavelength selected should be as near as possible to that for the peak of the luminosity curve to take advantage of the maximum sensitivity of the eye. Likewise, an inspection of the curve coordinating concentration and transmittancy shows that certain portions will yield results of greater accuracy than others. Readings in the optimum range can be obtained by suitable dilution of the sample, or by changing cell thickness.

Later, Mehlig used a modified method for calculating results in applying the method to the determination of copper in ores and mattes *(135)*. For solutions conforming to the Bouguer-Beer equation

$$I = I_0 \times 10^{-Kbc}$$

in which $I_0$ represents the intensity of the incident light of given wavelength entering the solution, $I$ the intensity on leaving the solution, $b$ the length of the absorption cell in centimeters, $c$ the moles of absorbing substance per liter of solution, and $K$ the molecular extinction coefficient. Solving for $c$, we have

$$c = \frac{\log_{10} I_0/I}{Kb} \text{ moles per liter}$$

The value for $b$ is known and $I/I_0$ is the transmittancy. It is necessary to prepare a solution of known concentration, $c$, in order to calculate the value of $K$ for desired wavelengths for use in subsequent work. As in the use of a transmittancy-wavelength curve, the wavelengths selected should be those of the optimum portion of the transmittancy curve.

Applying this procedure to the determination of manganese as permanganate (Fig. 38, curve 4), the transmittancy $I/I_0$ is 0.140 (14.0 per cent) at a wavelength of 525 mμ. Since the concentration $c$ is 10.00 mg. (0.0001825 moles) of manganese per liter, and the cell thickness $b$ is 2.00 cm., the constant $K$ is 2339 for the curve determined at 5 mμ band width. Once this constant is calculated at a



given wavelength for a solution conforming to Beer's law, it may then be used under the same conditions in calculating concentrations of solutions of unknown value. In general, systems for which the value of $K$ is large are preferable *(12)*.

In connection with his work on copper ores and mattes Mehlig reached the conclusion that the spectrophotometric method gave results in this case as satisfactory as the titrimetric iodide method and that the data were obtained more rapidly and more conveniently. Some of his results are shown in Table V.

TABLE V.

*Spectrophotometric Determination of Copper*

| Spectrophotometric Method | | | | Iodide Method | Difference |
|---|---|---|---|---|---|
| Transmittancy Measured at | | | Average | | |
| 570 mμ | 580 mμ | 590 mμ | | | |
| % | % | % | % | % | % |
| 7.33 | 7.27 | 7.24 | 7.28 | 7.27 | +0.01 |
| 6.27 | 6.27 | 6.29 | 6.28 | 6.27 | +0.01 |
| 5.37 | 5.42 | 5.43 | 5.41 | 5.37 | +0.04 |
| 3.98 | 3.93 | 3.90 | 3.94 | 3.94 | 0.00 |
| 3.09 | 3.09 | 3.08 | 3.09 | 3.03 | +0.06 |
| 8.55 | 8.52 | 8.44 | 8.50 | 8.56 | —0.06 |
| 8.27 | 8.26 | 8.25 | 8.26 | 8.22 | +0.04 |
| 14.16 | 14.12 | 14.07 | 14.12 | 14.09 | +0.03 |
| 2.22 | 2.20 | 2.17 | 2.20 | 2.24 | —0.04 |
| 4.30 | 4.29 | 4.27 | 4.29 | 4.34 | —0.05 |
| 21.80 | 21.61 | 21.61 | 21.67 | 21.61 | +0.06 |

It should be noted that these values ranged from 2 to 21 per cent. Later *(136)* Mehlig determined iron in ores ranging from 36 to 57 per cent. This is noteworthy in view of the general belief that colorimetric determinations are limited to maximum concentrations of a few parts per million of the desired constituent. In this connection the work of Drabkin and Austin is especially interesting in that they report *(43)* determining hemoglobin over a range of 0.00003 to 25.58 mM/L.

b.' **Two (or more) Constituents.** Spectrophotometers, especially those operating on narrow band widths, have their most distinctive analytical value for systems containing two, or more, color components.

In such systems three situations, more or less distinct, may arise. In the first the spectral transmittancy curves of the two components are such that the transmittancy for one of the desired constituents

may be determined by taking readings at a wavelength where the second constituent does not interfere (its transmittancy is 100 per cent). In the second the readings have to be made at a wavelength where both constituents contribute to the absorption, but it is possible to correct for the effect of one of them by means of readings at a different wavelength. These two are illustrated by the curves in Fig. 46 for a mixture of permanganate and dichromate *(192)*. Silverthorn and Curtis recommend taking transmittancy readings for the permanganate alone at 575 m$\mu$, where the value for the dichromate is practically 100 per cent, and for the mixture at 450 m$\mu$. The latter value must then be corrected for the amount of permanganate found for this constituent alone.

In the third situation the curves for the constituents of the mixture are such that the preceding possibilities are inapplicable. In general, solution of this problem depends upon using equations involving transmittancies of each component and of the mixture at properly selected wavelengths.

An example of this kind of application is the work of Comar and Zscheile *(33)* in determining $\alpha$- and $\beta$-chlorophylls in mixtures of the two. Fig. 47 shows the absorption spectra of the pure chlorophylls plotted in terms of the specific extinction coefficient $k$ (designated by the authors as absorption coefficient $a$). Beer's law was used in the form

$$k = \frac{\log_{10} 1/T}{b\,c}$$

with the various symbols having their previously specified significance.

In considering solutions in which two (or more) components contribute to the absorption at the wavelengths employed, the values of $\log_{10} 1/T$ and of $kbc$ for the individual components are additive. The extinction coefficient, $k$, is an intensive property and is not additive. Thus, for a given wavelength,

$$\log_{10} 1/T_{(\text{observed})} = \log_{10} 1/T_{(1)} + \log_{10} 1/T_{(2)} = k_1 b_1 c_1 + k_2 b_2 c_2$$

Values for the concentration of each of $n$ components may be obtained from transmittancy measurements at $n$ wavelengths, where no two curves coincide or intersect; however, no two wavelengths may be used at which transmittancy values for two components have the same ratio. This is the general case.

For the two component system of the chlorophylls, where $b$ remains constant, the equations are simplified as follows:

$$\begin{array}{ll} \text{At wavelength } \lambda' & \log_{10} 1/T = (k_1' c_1 + k_2' c_2)\, b \\ \text{At wavelength } \lambda'' & \log_{10} 1/T = (k_1'' c_1 + k_2'' c_2)\, b \end{array}$$



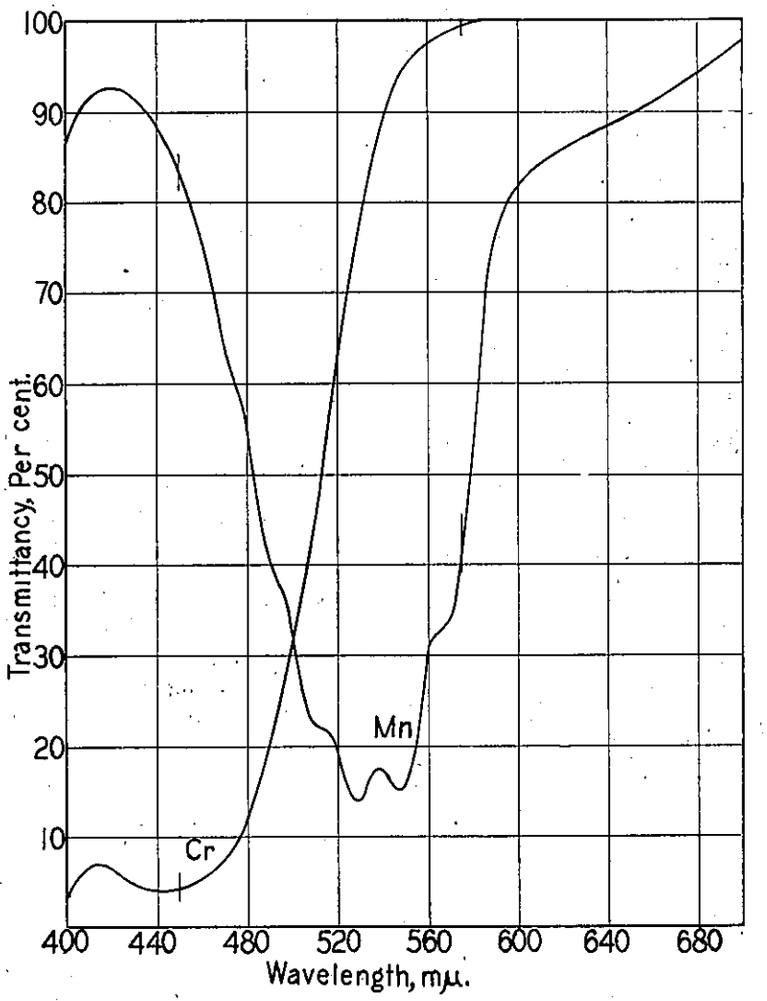



FIG. 46. Transmittancy curves for acidic solutions of potassium permanganate and potassium dichromate.

where $\log_{10} 1/T$ = the measured value at the given wavelength; $k_1'$ and $k_2'$ = the specific extinction coefficients* of components 1 and 2, respectively, at wavelength $\lambda'$; $k_1''$ and $k_2''$ = the specific extinction coefficients of components 1 and 2, respectively, at wavelength $\lambda''$; and $c_1$ and $c_2$ = the concentrations of components 1 and 2, respectively, in grams per liter. These two equations may be solved simultaneously to obtain values of the two unknowns, $c_1$ and $c_2$.

The total concentration $C$, in grams per liter, can be obtained also from determination of $\log_{10} 1/T$ at a coincident point for the two curves. Then the general equation reduces to

$$\log_{10} 1/T = kbC$$

in which $C = c_1 + c_2$. If the total concentration is known, the composition may be determined by measurement of the transmittancy at one suitable non-coincident point. Such a solution would involve use of the simultaneous equation just given, along with the general one given first.

Maximum analytical accuracy is achieved when the relative heights of the curves at the two wavelengths are reversed for the two components. Inaccuracies are introduced easily when readings are taken on steep slopes. In general, the accuracy is improved with an increase in the distance between the two curves at the wavelength employed. Thus, at wavelengths 5460 and 5890 Å the analytical results are extremely sensitive to light-absorbing impurities.

In their work on chlorophylls, based on the curves in Fig. 47, Comar and Zscheile used transmittances determined at the following wavelengths: 6425 and 6600 for total chlorophyll and for percentage composition; 5680, 5810, and 6000 for checks on total chlorophyll; and 5460, 5890, and 6130 for checks on percentage composition. In a similar manner, Zscheile and Beadle *(249)* determined $\beta$-carotene and neo-$\beta$-carotene by means of a visual spectrophotometer.

Variations and extensions of the method are given in other sources *(12, 13, 42, 128, 149, 154, 179, 214, 225)*. Thus, in addition to using this kind of method for such binary systems, Miller *(148)* has extended it to ternary and quaternary systems. Weigert reported the analysis of a four-component system of dyes *(226)*. As is generally the case with indirect determinations, the reliability of results for such mixtures is not all that one might wish, even with the most carefully determined absorptances. Where such methods are applied to mixtures, however, there is usually no satisfactory alternative.

* NOTE: In Chapter 4 the character $k'$ was used for $\log_e$ values. Here the "primes" simply differentiate between constants obtained at different wavelengths.



To reduce the time required for the calculations involved with multicomponent systems, an electrical "spectrocomputer" is now available *(47a)*.

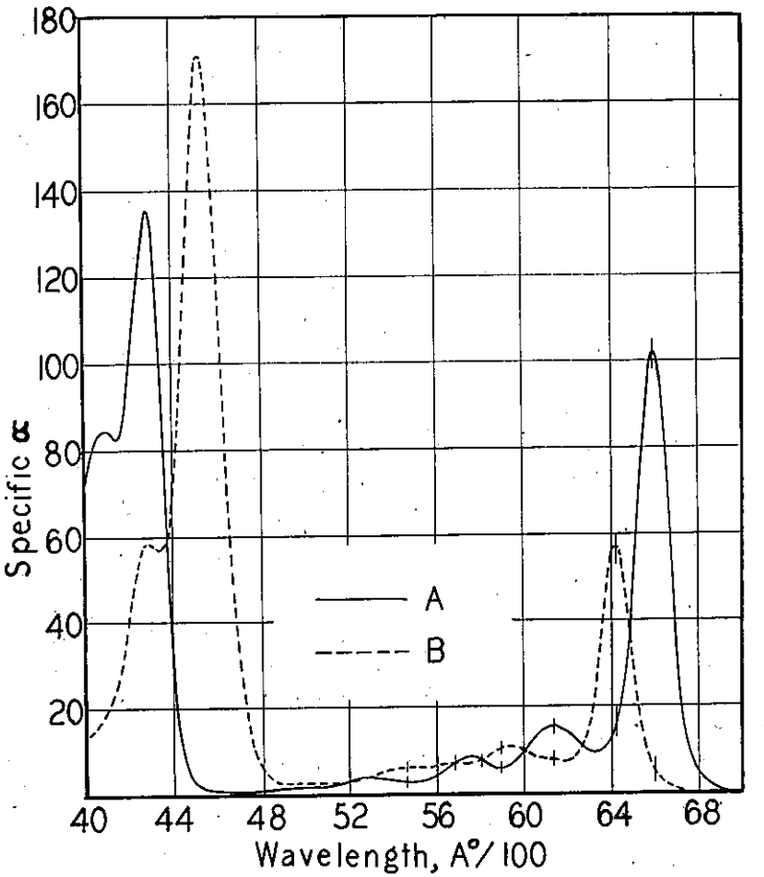



FIG. 47. Absorption spectra of chlorophylls A and B.

Perhaps a word of caution should be directed to the novice in spectrophotometry. With the best of modern instruments, a competent operator can determine absorptance data satisfactorily. The question is whether the system was prepared so that the measurement is worth making. Morton *(154)* has summarized the formidable difficulties encountered in applying the absorptometric method to vitamin A in the ultraviolet region. Similar problems arise in colorimetry, as pointed out by Zscheile and Comar in their discussion of the influence of the preparative procedure on the purity of chlorophyll components *(250)*.

d. **Color Analysis.** In the discussion of stimulimeters it was stated that such instruments are used primarily for measuring color as color. That is, the determination gives a colorimetric specification, in terms of equivalent stimuli, rather than the amount of a desired constituent. Although stimulimeters have their place for this purpose, especially in certain industrial applications, physicists agree that spectrophotometric curves provide the fundamental basis for specifying a color in terms of the radiant energy evoking the sensation.

The present discussion of this phase of spectrophotometry includes little beyond a statement of definitions and an outline of methods of calculation. The *Handbook of Colorimetry*, by A. C. Hardy *(83)*, should be consulted for a discussion of the physics of the subject, and for the accompanying tables and graphs which are a prerequisite for rapid, reliable application of the methods. In order to prevent confusion when referring to his publication, the same symbols are used here. The Committee on Colorimetry has devoted part of its report to this subject *(34d)*.

As already stated, the data yielded directly by a spectrophotometer consist of single values of transmittance (or reflectance) at given wavelengths, or a graph, such as a transmittancy-wavelength curve, incorporating these values for a certain spectral region.

To one experienced in spectrophotometric colorimetry the transmittance-wavelength curve itself has considerable qualitative and some quantitative value as a specification of color *(58)*. In Fig. 38 the high transmittancies in the red and the violet regions, and the low values in the green, indicate that a purple hue should result from the combination of red and violet. Similarly, a system with low transmittancies below 500 mμ, and only high values above this wavelength, should have a yellow hue. In addition to the hue of the permanganate system, the curves in Fig. 38 indicate something concerning the relative concentrations of the solutions. Thus, the top curve, with high transmittancies throughout, indicates a dilute solution for a solute of high absorptive capacity; and the bottom curve, with its deep absorption band, applies to a solution of much deeper color (and greater concentration). In a sense, curves such as these represent a numerical specification in terms of intensity and wavelength factors. This is a purely physical designation of the characteristics of the radiant energy transmitted. Increasing attention is being given to this aspect of spectrophotometry in industrial work *(5, 232)*.

**Psychophysical Specification.** In order to specify a color in terms of the stimuli which evoke the sensation experienced on viewing the



system in question, it is necessary to determine the combination of known stimuli which will color match the system. Two schemes are in use for this purpose. In the trichromatic system one determines, in percentages,* the amounts of three primary stimuli, red, green,

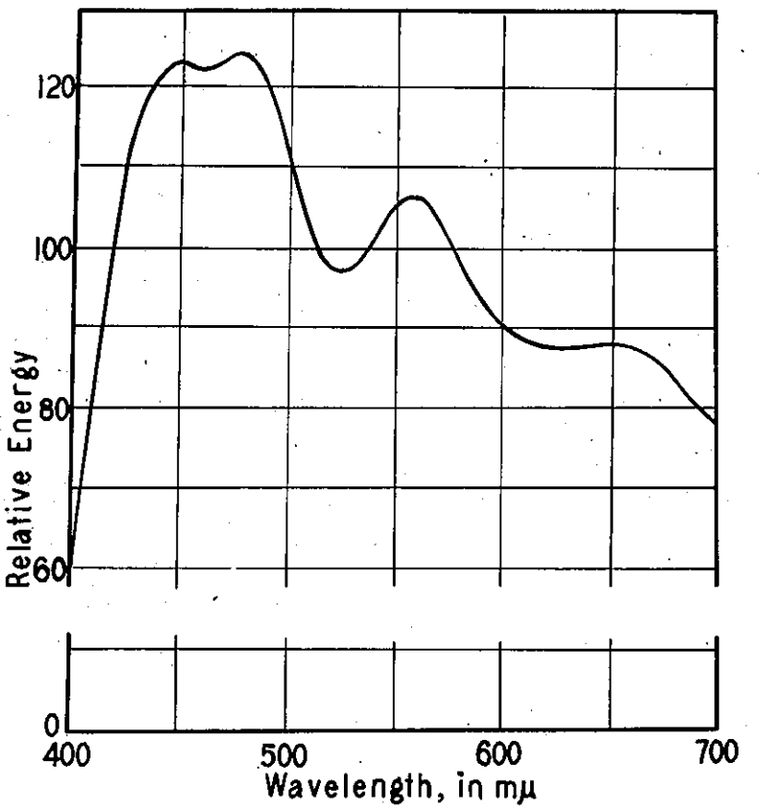



FIG. 48. Relative spectral distribution of the energy radiated per unit time by I.C.I. illuminant C.

and violet, required to match the color. In the monochromatic system the specification is calculated in terms of dominant wavelength, in millimicrons, and purity and luminance, in percentages. These two possibilities will now be considered briefly.

a.' **Trichromatic System.** Since the color sensation experienced on viewing a colored system depends both upon the color vision of the

* NOTE: Hardy (*Handbook of Colorimetry*) uses decimal fractions, called trichromatic coefficients.

observer and upon the spectral energy distribution of the source of illumination, it is necessary in specification work to adopt standards for each. This was done in 1931 by the International Commission on Illumination *(79, 105)* which agreed upon what is now referred to as the I.C.I. standard observer and the standard illuminants *A*, *B*, and *C*.

The standard illuminant with which this discussion is concerned, known as I.C.I. illuminant *C*, is a close approximation to average daylight *(34d)*. Fig. 48 shows the relative spectral distribution of the energy radiated per unit time by this light source.

It is sufficient here to note that the standard observer was taken as an average of the response characteristics of a group of individuals found to possess normal color vision. Based upon this work, the data in Fig. 49 show the tristimulus values of this standard observer for the various spectrum colors. The values of $\bar{x}$ (red), $\bar{y}$ (green)*, and $\bar{z}$ (violet) are the amounts of the three I.C.I. primaries required to color match a unit amount of energy having the indicated wavelength.

The values calculated hereafter, based upon the data in Figs. 48 and 49, may be considered, then, as the characteristics of the color as viewed by an observer with normal color vision when the system is illuminated with average daylight.

To make such calculations one needs, in addition to the data in Figs. 48 and 49, the spectral transmittance (or reflectance) curve for the system concerned. For the present purpose the curve for 4 p.p.m. of iron has been taken from Fig. 17. Two variations in the method of calculation will be considered for dealing with the data of this curve.

a." **Weighted Ordinate Method.** If one multiplies, at any given wavelength, the ordinate for illuminant *C* ($E_C$) by the transmittancy *(T)* of the sample, the product, $TE_C$, is the relative energy stimulus for this wavelength. Then on multiplying $TE_C$ by the corresponding ordinates for $\bar{x}$, $\bar{y}$, and $\bar{z}$, one obtains $TE_C\bar{x}$, $TE_C\bar{y}$, and $TE_C\bar{z}$. Since $E_C$ and $\bar{x}$, $\bar{y}$, and $\bar{z}$ are constants, their products, at wavelength intervals of 10 m$\mu$, are given in Table VI. The values are taken from Hardy's *Handbook of Colorimetry*, Table XV, p. 45.*

Table VII contains the calculated values $TE_C\bar{x}$, $TE_C\bar{y}$, and $TE_C\bar{z}$ for the curve in Fig. 17. These data show the contribution, at the respective wavelengths, of each primary to the total stimulation.

* NOTE: Figures 48, 49, and 50 and Tables VI and VIII are reprinted with permission from *Handbook of Colorimetry*, copyright 1936 by the Massachusetts Institute of Technology.

* NOTE: The $\bar{y}$ curve corresponds with the relative luminosity curve of the human eye.



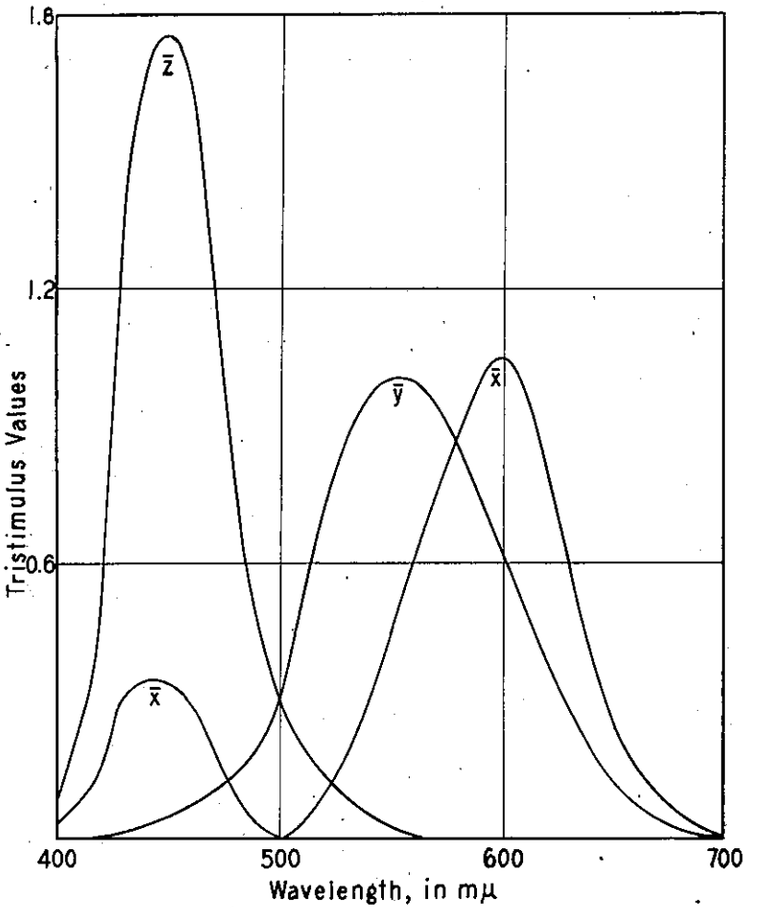



FIG. 49. Tristimulus values for the various spectrum colors. The values of $\bar{x}$, $\bar{y}$, $\bar{z}$, are the amounts of the three I.C.I. primaries required to color match a unit amount of energy having the indicated wavelength.

TABLE VI.

*Tristimulus Values for Spectrum Colors, Weighted by Energy Distribution of I.C.I. Illuminant C.*

| Wavelength | $E_C\bar{x}$ | $E_C\bar{y}$ | $E_C\bar{z}$ |
|---|---|---|---|
| 400 | 0.91 | 0.03 | 4.34 |
| 410 | 3.48 | 0.10 | 16.58 |
| 420 | 13.19 | 0.39 | 63.36 |
| 430 | 31.92 | 1.30 | 155.74 |
| 440 | 42.15 | 2.79 | 211.43 |
| 450 | 41.69 | 4.71 | 219.81 |
| 460 | 35.81 | 7.39 | 205.49 |
| 470 | 24.19 | 11.27 | 159.46 |
| 480 | 11.85 | 17.22 | 100.71 |
| 490 | 3.86 | 25.11 | 56.13 |
| 500 | 0.55 | 36.21 | 30.49 |
| 510 | 0.95 | 51.46 | 16.18 |
| 520 | 6.13 | 68.80 | 7.58 |
| 530 | 16.22 | 84.48 | 4.13 |
| 540 | 29.65 | 97.40 | 2.08 |
| 550 | 45.60 | 104.67 | 0.92 |
| 560 | 62.60 | 104.77 | 0.41 |
| 570 | 77.97 | 97.39 | 0.21 |
| 580 | 89.61 | 85.09 | 0.15 |
| 590 | 95.68 | 70.55 | 0.11 |
| 600 | 95.26 | 56.60 | 0.07 |
| 610 | 88.65 | 44.47 | 0.03 |
| 620 | 75.28 | 33.57 | 0.02 |
| 630 | 56.54 | 23.32 | 0.01 |
| 640 | 39.32 | 15.37 | 0.00 |
| 650 | 25.01 | 9.44 | ..... |
| 660 | 14.50 | 5.36 | ..... |
| 670 | 7.54 | 2.76 | ..... |
| 680 | 3.93 | 1.43 | ..... |
| 690 | 1.82 | 0.66 | ..... |
| 700 | 0.87 | 0.31 | ..... |

After obtaining the products shown in Table VII, the next operation is to add each calculated column. This gives the three tristimulus values, $X$, $Y$, and $Z$. Dividing the value of each of these by the sum of the three $(X+Y+Z)$ gives the three trichromatic coefficients, $x$, $y$, and $z$. The latter become, respectively, the percentages of red, green, and violet on multiplying by 100. The calculations involved make this method somewhat tedious to use.

b." **Selected Ordinate Method.** This is the shorter of the two alternative methods of calculation. In contrast to the weighted ordinate method, the transmittance (or reflectance) values are read at the



TABLE VII.

*Trichromatic Data for Iron Solution by the Weighted Ordinate Method*

| Wavelength | Transmittancy | $TE_C\bar{x}$ | $TE_C\bar{y}$ | $TE_C\bar{z}$ |
|---|---|---|---|---|
| Mμ | % | | | |
| 400 | 49.0 | 45 | 1 | 213 |
| 410 | 42.0 | 146 | 4 | 696 |
| 420 | 36.5 | 481 | 14 | 2313 |
| 430 | 32.3 | 1031 | 42 | 5030 |
| 440 | 29.2 | 1231 | 82 | 6174 |
| 450 | 26.5 | 1105 | 125 | 5825 |
| 460 | 23.5 | 842 | 174 | 4829 |
| 470 | 21.0 | 508 | 237 | 3349 |
| 480 | 20.0 | 237 | 344 | 2014 |
| 490 | 19.5 | 75 | 490 | 1095 |
| 500 | 18.0 | 10 | 652 | 549 |
| 510 | 18.0 | 17 | 926 | 291 |
| 520 | 20.7 | 127 | 1424 | 157 |
| 530 | 28.5 | 462 | 2408 | 118 |
| 540 | 44.0 | 1305 | 4286 | 92 |
| 550 | 64.0 | 2918 | 6699 | 59 |
| 560 | 77.0 | 4820 | 8067 | 32 |
| 570 | 86.0 | 6705 | 8376 | 18 |
| 580 | 91.0 | 8155 | 7743 | 14 |
| 590 | 94.7 | 9061 | 6681 | 10 |
| 600 | 97.0 | 9240 | 5490 | 7 |
| 610 | 98.7 | 8750 | 4389 | 3 |
| 620 | 99.7 | 7505 | 3347 | 2 |
| 630 | 100.0 | 5654 | 2332 | 1 |
| 640 | 100.0 | 3932 | 1537 | ..... |
| 650 | 100.0 | 2501 | 944 | ..... |
| 660 | 100.0 | 1450 | 536 | ..... |
| 670 | 100.0 | 754 | 276 | ..... |
| 680 | 100.0 | 393 | 143 | ..... |
| 690 | 100.0 | 182 | 66 | ..... |
| 700 | 100.0 | 87 | 31 | ..... |
| ..... | ..... | 79729 | 67866 | 32891 |
| ..... | ..... | 44.2% (Red) | 37.6% (Green) | 18.2% (Violet) |

Dominant wavelength = 588.6 mμ; purity = 51.5%; luminance = 63.8%.

wavelengths shown in Table VIII. For most purposes these 30 wavelengths are sufficient (*83*, p. 51), although Hardy has included values for 100 wavelengths in the *Handbook of Colorimetry*. For relatively non-selective curves the starred wavelengths are often adequate.*

* NOTE: The wavelengths were selected in such a way that they mark the median of the equal-energy areas underneath the curves obtained by multiplying the ordinates of the I.C.I. primaries (Fig. 49) by the corresponding ordinates of Illuminant *C* (Fig. 48).

TABLE VIII.

*Selected Ordinates for Illuminant C*

| Number | X | Y | Z |
|---|---|---|---|
| 1 | 424.4 | 465.9 | 414.1 |
| 2* | 435.5* | 489.4* | 422.2* |
| 3 | 443.9 | 500.4 | 426.3 |
| 4 | 452.1 | 508.7 | 429.4 |
| 5* | 461.2* | 515.1* | 432.0* |
| 6 | 473.9 | 520.6 | 434.3 |
| 7 | 531.0 | 525.4 | 436.5 |
| 8* | 544.2* | 529.8* | 438.6* |
| 9 | 552.3 | 533.9 | 440.6 |
| 10 | 558.7 | 537.7 | 442.5 |
| 11* | 564.0* | 541.4* | 444.4* |
| 12 | 568.8 | 544.9 | 446.3 |
| 13 | 573.2 | 548.3 | 448.2 |
| 14* | 577.3* | 551.7* | 450.2* |
| 15 | 581.2 | 555.1 | 452.1 |
| 16 | 585.0 | 558.5 | 454.0 |
| 17* | 588.7* | 561.9* | 455.9* |
| 18 | 592.3 | 565.3 | 457.9 |
| 19 | 595.9 | 568.8 | 459.9 |
| 20* | 599.5* | 572.5* | 462.0* |
| 21 | 603.2 | 576.4 | 464.1 |
| 22 | 606.9 | 580.4 | 466.4 |
| 23* | 610.8* | 584.8* | 468.8* |
| 24 | 614.9 | 589.5 | 471.4 |
| 25 | 619.2 | 594.8 | 474.4 |
| 26* | 624.0* | 600.8* | 477.8* |
| 27 | 629.6 | 607.6 | 481.9 |
| 28 | 636.4 | 616.0 | 487.3 |
| 29* | 646.2* | 627.1* | 495.3* |
| 30 | 662.2 | 647.0 | 511.5 |
| Factors | | | |
| 30 ordinates | 0.03265 | 0.03333 | 0.03937 |
| 10 ordinates | 0.09804 | 0.10000 | 0.11812 |

In using this method the primary operation consists in adding, for each of the three primaries, the ordinates of the transmittance (or reflectance) curve at the selected wavelengths, and multiplying each sum by the appropriate factor, as shown in Table VIII, to obtain the tristimulus values, *X*, *Y*, and *Z*. The trichromatic coefficients are then obtained as before. Data for the curve in Fig. 17 are shown in Table IX.



TABLE IX.

*Trichromatic Data for Iron Solution by the Selected Ordinate Method*

| Ordinate Number | X | Y | Z |
|---|---|---|---|
| 1 | 34.5 | 21.7 | 39.7 |
| 2 | 30.6 | 19.4 | 35.3 |
| 3 | 28.0 | 18.0 | 34.0 |
| 4 | 26.0 | 17.8 | 32.5 |
| 5 | 23.2 | 18.8 | 31.7 |
| 6 | 20.5 | 21.0 | 31.0 |
| 7 | 29.5 | 24.0 | 30.0 |
| 8 | 53.0 | 29.0 | 29.7 |
| 9 | 66.5 | 34.0 | 29.0 |
| 10 | 76.0 | 40.0 | 28.5 |
| 11 | 81.5 | 48.0 | 28.0 |
| 12 | 85.5 | 55.0 | 27.5 |
| 13 | 88.0 | 61.0 | 27.0 |
| 14 | 90.0 | 67.0 | 26.5 |
| 15 | 91.5 | 71.0 | 26.0 |
| 16 | 93.0 | 75.0 | 25.5 |
| 17 | 94.5 | 79.0 | 24.8 |
| 18 | 95.3 | 82.5 | 24.2 |
| 19 | 96.2 | 85.5 | 23.5 |
| 20 | 97.0 | 87.5 | 22.8 |
| 21 | 97.7 | 89.5 | 22.2 |
| 22 | 98.3 | 91.2 | 21.5 |
| 23 | 98.7 | 93.0 | 21.0 |
| 24 | 99.3 | 94.8 | 20.6 |
| 25 | 99.7 | 96.0 | 20.3 |
| 26 | 100.0 | 97.2 | 20.0 |
| 27 | 100.0 | 98.3 | 19.8 |
| 28 | 100.0 | 99.3 | 19.5 |
| 29 | 100.0 | 100.0 | 18.8 |
| 30 | 100.0 | 100.0 | 18.0 |
| .... | 2294.0 | 1914.5 | 778.9 |
| Times Multiplying Factors | 74.90 | 63.81 | 30.66 |
| | 44.2% | 37.6% | 18.2% |

In order to simplify this method still more, a manual tristimulus calculator is very useful *(207)*. Van den Akker designed a mechanical integrator *(216)*. Spencer*(199)* has proposed a calculation for introducing the adaptation of the eye into the color specification system.

The tristimulus data, calculated as tristimulus coefficients or as percentages of the three primaries, red, green, and violet, are often

an adequate numerical specification of color. In case luminance is also desired, it may be obtained as indicated in the next section.

b.' **Monochromatic System.** For some purposes it may be desirable to specify a color in terms of the monochromatic terms, dominant wavelength, in millimicrons, and purity and luminance, in percentages. Respectively, these terms may be taken as a measure of the psychological attributes, hue, saturation, and brightness.

**Luminance.** This value is a measure of the percentage of the visual radiant energy incident on the sample that is transmitted (or reflected) by it.

To obtain the luminance, using the weighted ordinate method for calculating tristimulus values, one divides the total for the $Y$ (green) column in Table IX by 1065* and multiplies by 100. This gives 63.8 for the system chosen. In the selected ordinate method the tristimulus value $Y$ (green), when multiplied by 100, gives the value directly, as shown in Table IX.

**Dominant Wavelength and Purity.** Dominant wavelength may be defined as the wavelength of the spectral color which would match the sample when diluted with the light source used, in this case illuminant $C$. The purity is the difference between 100 and the percentage of illuminant $C$ used for the dilution.

In determining dominant wavelength and purity use is made of the coefficients calculated for the trichromatic system, and of the color or chromaticity diagram shown in Fig. 50. Trichromatic coefficients $x$ (red) and $y$ (green) form the coordinate values for this graph. The center $C$ of the elliptical figure marks the coordinate $(x, y)$ values of illuminant $C$. The elliptical border line is the locus of the spectrum colors, the wavelengths of which are marked by the lines radiating from the center.

With the elliptical border line representing pure (100 per cent) spectral colors, and the center $C$ the point of zero purity, the intermediate elliptical lines mark degrees of purity between zero and 100 per cent.

To determine dominant wavelength, locate the point in the color diagram marking the intersection of perpendiculars from the $x$ (red) and $y$ (green) trichromatic coefficients. Through this point, and from the central point $C$, project a straight line to the border line. The point of intersection of the radial line with the border line marks the dominant wavelength. The purity is determined by noting the

* NOTE: This value is the sum of $E_0\bar{y}$ values in Table VI, and assumes a transmittance of 100 per cent throughout the wavelength range.



position of the $x$, $y$ point with respect to the various elliptical lines. For more reliable values, one must use the larger charts contained in Hardy's *Handbook of Colorimetry*.

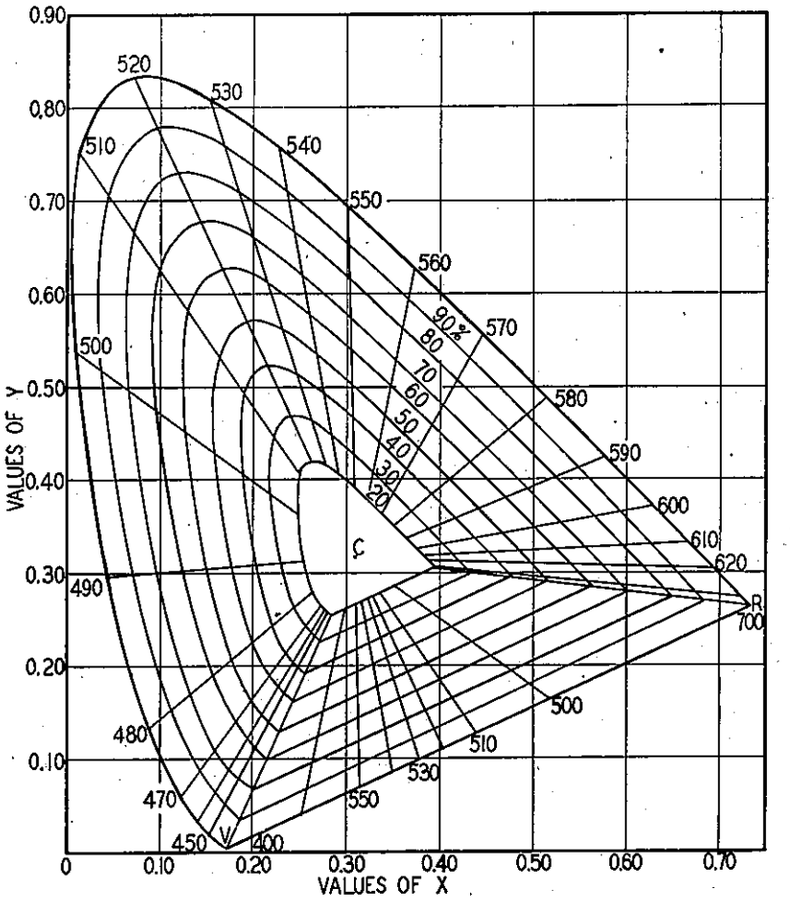



FIG. 50. Chromaticity diagram.

The portion of the color diagram indicated by the area $VCR$ includes the purples. Since purples are mixtures of red and violet, they have no dominant wavelength. For $x$, $y$ points falling in this area it is conventional to designate the wavelength of the complementary color. This is accomplished by drawing a straight line from the $x$, $y$ point through $C$ to the intersection with the elliptical border line. The value located is marked as this wavelength, followed by the letter $c$ to show that it is a complementary value.

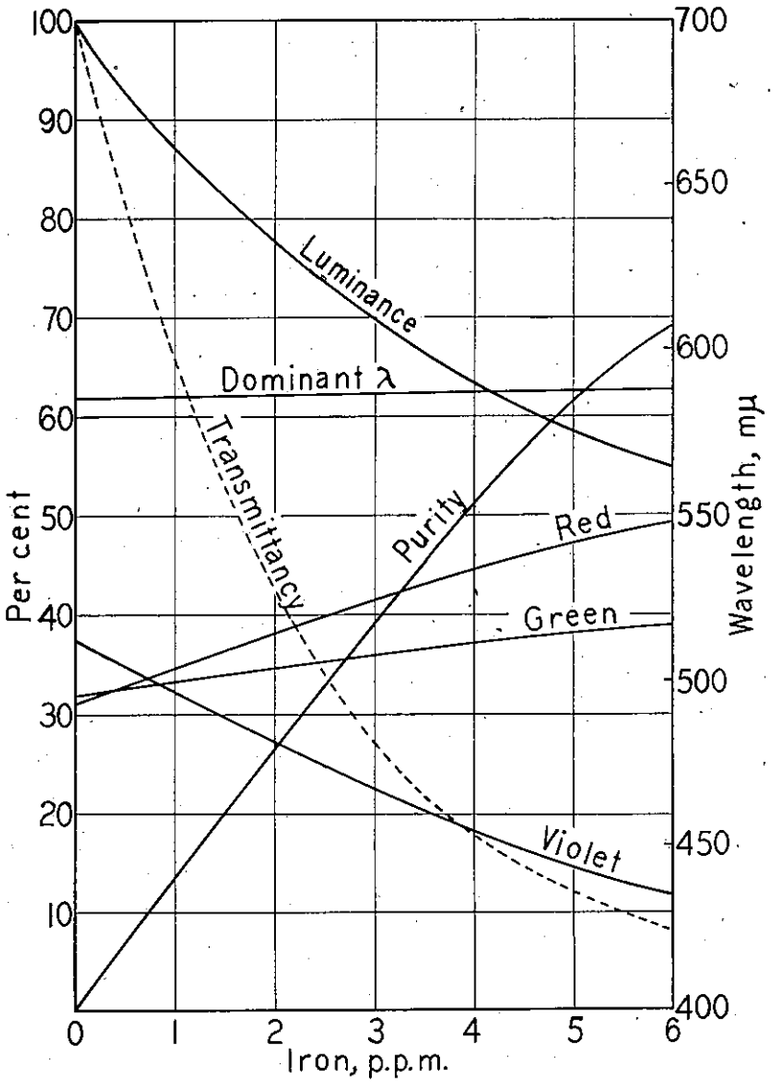



FIG. 51. Color analysis data for solutions of 1,10-phenanthroline-iron complex.



Fig. 51 shows curves coordinating concentration with the trichromatic and monochromatic color analytical data calculated for the curves contained in Fig. 17.

**Uses of Numerical Specifications.** Current chemical publications are almost devoid of references to "color analyses." Apparently chemists have made little use of such data, outside of the rather general application for specification of color, which has been primarily for reflectance measurements on opaque materials.

So little has been accomplished thus far to bring these numerical evaluations of colorimetric characteristics into everyday use in analysis and testing that one can hardly predict to what extent they will become a part of our general thinking in the near future. Undoubtedly, the invention of rapid, reliable spectrophotometers and the simplification of the necessary calculations will do much to extend the application of such data. When the transmittance (or reflectance) curve itself is inadequate, this is the fundamental method.

Such numerical values represent a kind of color language which is more definite than any system based upon verbal description or upon samples of colored materials. The calculated values provide a specification from which one can form an idea of the color without seeing it. A few examples will illustrate the possibilities.

Hardy *(83)* has listed the values for a number of colors commonly specified by name. Pineo pointed out *(168)* the value of trichromatic characteristics in measuring color fading, in color matching, and in dyeing control, and Shelton and Emerson *(191)* commented on the possibility of using such data for dyed fabrics. Moon has published data for many architectural materials *(153)*. Recently colors recommended for use in industrial safety marking have been designated *(46)* in trichromatic terms (along with Munsell and Ostwald designations). Such measurement is now a tentative method of the American Society for Testing Materials *(6)*, and "is intended to determine the spectral characteristics of light-transmitting and light-reflecting objects and materials, and to designate their colors." Similarly, the American Standards Association has approved this method *(7)*. Since 1934 the colors of the flag of the United States have been specified in terms of trichromatic values *(162)*.

Related to this type of application is the suggestion of methods for computing the formulation of colorants needed to effect a visual color match of a given standard from spectrophotometric measurements of the colorants and the standard *(167)*. Saunderson *(185)* has used spectrophotometric data for the calculation of the color of pigment mixtures involving color matches.

# CHAPTER 6

## EXPERIMENTS IN SPECTROPHOTOMETRY

In recent years, especially since the introduction of photoelectric instruments, there has been a greatly increased interest in the application of spectrophotometry to colorimetric problems. It has been estimated that more than a million curves are being determined annually in industrial laboratories, and this number is increasing rapidly.

In order to provide a variety of experience in obtaining and using spectrophotometric data *(141)*, various colored solutions may be selected from those available. The preferable systems depend upon the characteristics which are to be studied, since many solutions differ widely in colorimetric properties. For some experiments, such as that showing the effect of spectral band width, suitable transparent solids are just as satisfactory. Opaque solids, such as plastics and textiles, may be used for color analysis measurement.

**Apparatus.** Any of the better spectrophotometers, either visual or photoelectric, may be used. Readings with visual instruments are increasingly unreliable below 440 or above 670 m$\mu$ unless monochromatic light sources are used. This results from the low sensitivity of the normal eye in these regions. If several different types of instruments are available, it is instructive to make a comparative study with them to determine the relative accuracy, time required, ease of operation, and other factors.

Since the technic of operating a spectrophotometer depends upon the instrument, instructions should be available for the one to be used.

In measuring solutions, the absorption cell should be carefully cleaned and finally rinsed with some of the solution to be measured. After filling the cell, the outside faces through which the light beam passes should be wiped dry and clean with absorbent tissue. If two cells are used, the second will contain the solvent only. Unless otherwise specified, 1 cm. cells are assumed.

**Solutions.** The following stock solutions, prepared as noted, may be used:

*Potassium Permanganate.* Use a solution of known concentration to prepare 500 ml. of one containing 50 mg. of manganese per liter.



*Potassium Dichromate.* Prepare 1000 ml. of a 0.1 M. solution by dissolving material of known purity in water.

*Iron.* Dissolve 0.1000 gm. of iron wire, of reagent quality, in a small amount of hydrochloric acid (1:4). To insure oxidation of the iron, add some hydrogen peroxide and boil out the excess. Dilute the solution to 1000 ml. Appropriate dilutions may then be made from this stock solution which contains 100 mg. of iron per liter (100 p.p.m).

**Study of Significant Factors.** Some of the factors which may be encountered, either with spectrophotometers as instruments or with the solutions being measured, are illustrated in the exercises described herewith. The different solutions suggested will show the variety of colorimetric properties desired.

Spectrophotometric data, in the form of curves, may be presented in several ways, the one used depending upon the instrumental readings and the purpose for which the data are obtained. It is assumed here that the readings will be transmittancy (or reflectance), in per cent, and wavelength, in millimicrons. Other systems can then be calculated from these.

a. **Effect of Concentration.** Prepare 50 ml. each of a series of permanganate solutions having concentrations of 50, 35, 20, 10, 5, 2.5, 1.0, and 0.25 mg. of manganese per liter. With the spectrophotometer set for a definite spectral band width, such as 10 m$\mu$, determine the transmittancy-wavelength curve for each solution by taking transmittancy readings at intervals of 10 m$\mu$ from 400 to 700 m$\mu$.

Plot the series of curves with transmittancy as ordinate and wavelength as abscissa, as shown in Fig. 38. Taking 95 and 5 per cent as the upper and lower limits, respectively, for reliable measurement at the peak of the absorption band, what is the range of concentration that may be measured in a 1-cm. cell?

At least one of these curves should be plotted in each of the other forms often used. Other ordinates are optical density, $D$, ($= \log 1/T$), or log $D$, on a linear scale, and transmittancy ($= T$) on a log scale; other abscissas are log wavelength, or frequency ($=3 \times 10^5$/wavelength, in m$\mu$), on a linear scale, and wavelength, on a log scale.

A curve shape index may be constructed from the curve plotted with log $D$ as ordinate (linear scale) and log wavelength as abscissa (linear scale) *(193, 194)*.

b. **Effect of Cell Thickness.** Using the permanganate solution containing 5 mg. of manganese per liter, determine the transmittancies in cells of different thicknesses, such as 0.5, 1.0, 2.0, 5.0, and 10.0 cm., at intervals of 10 m$\mu$.

Plot the data in the form of a transmittancy-wavelength graph to determine the optimum thickness of cell for this concentration (that is, the thickness which gives a transmittancy of 15-30 per cent at the peak of the absorption band).

c. **Effect of Width of Spectral Band.** Determine the transmittancies of the permanganate solution containing 10 mg. of manganese per liter for different spectral band widths, preferably 2.5, 5.0, 10.0, and 20.0 m$\mu$, the readings being taken at intervals of 5 m$\mu$ from 480 to 580 m$\mu$.

Plot the data on a transmittancy-wavelength graph and note the difference in the curves, especially between 520 and 550 m$\mu$.

d. **Effect of Wavelength Intervals.** Determine the transmittancy of the permanganate solution containing 20 mg. of manganese per liter at a spectral band width of 10 m$\mu$ (or, better, 5 m$\mu$), the readings being taken at intervals of 10 m$\mu$ from 400 to 700 m$\mu$.

Plot three separate, smooth curves in the form of transmittancy-wavelength graphs by using the readings at intervals of 10, 20, and 40 m$\mu$ from 400 m$\mu$ upward. The effect here, as well as that for width of spectral band, is more striking for a solution of a neodymium salt or for a didymium glass (Corning No. 512). Comparison of the curve for the 10 m$\mu$ intervals with that obtained on a photometer with a set of 8-10 filters demonstrates one limitation of such instruments.

e. **Conformity of Solution to Bouguer's Law.** From the curves showing the effect of cell thickness tabulate the transmittancies for some given wavelength. Preferably this wavelength should be at a point of minimum transmittancy in the absorption band, which in the case of potassium permanganate is between 520 and 550 m$\mu$; otherwise select a wavelength where the transmittancy differences between different concentrations are large.

Using the transmittancy for some given cell thickness as a basis for calculation, such as that for 2 cm., calculate the transmittancy for the other measured thicknesses by means of the relationship,

$$T^{a/b} = T_1^{a/b_1}$$

in which $T$ is the transmittancy of the measured solution at a given wavelength, $b$ is its thickness, and $b_1$ is the thickness of the solution whose transmittancy $T_1$ is desired. Compare the calculated and observed transmittancies. The calculated values may be checked by means of a Keuffel and Esser color slide rule.

f. **Conformity of Solution to Beer's Law.** From the curves in Section "a" showing the effect of concentration of permanganate, tabulate the transmittancies, at the wavelength used in Section "e," for



the several concentrations. Construct a curve with concentration as the abscissa and $\log_{10} T$ as the ordinate, using linear scale paper; or on semilogarithmic paper plot $T$ directly as the ordinate and concentration as the abscissa on a linear scale. A straight line indicates conformity to Beer's law. If preferred, the optical density, as given directly on some instruments, may be plotted against concentration, using linear scales.

If conformity to Beer's law is found, calculate the molecular extinction coefficient $K$ for several concentrations by using the relationship,

$$K = \frac{\log_{10}(1/T)}{bc}$$

in which $T$ is the transmittancy for the wavelength used in Section "e," $b$ the thickness, and $c$ the concentration of the solute in moles per liter.

The exercise should be repeated with a solution such as potassium dichromate in water. Transmittancies for it should be determined for concentrations from 0.1 M. down to the limit of utility. The smaller concentrations are obtained by diluting the stock solution with water.

g. **Determination of the Amount of a Constituent.** If transmittancy-wavelength curves have been plotted for a series of concentrations of permanganate solutions (as in Section "a"), the data are at hand for determining manganese in steel spectrophotometrically. The solution of the unknown should be prepared according to accepted procedures (See Ref. *145*, p. 382).

Measure the transmittancy of the unknown solution with the spectrophotometer set to pass a 10 m$\mu$ spectral band whose median wavelength is one of the preferable values selected in Section "e." Two methods of calculating the concentration of the unknown may be tried.

In the first method a transmittancy-concentration curve is constructed from the data presented in Section "a" by plotting concentrations as abscissas and transmittancies, at the wavelength used for measuring the unknown, as ordinates. Then locate the measured transmittancy of the unknown on this curve and drop a perpendicular to the base line to find the desired concentration.

In the second method one solves for the unknown concentration $c$, in moles per liter, in the expression,

$$c = \frac{\log_{10}(1/T)}{Kb}$$

$T$ being the measured transmittancy, $K$ the molecular extinction

coefficient determined in Section "f," and $b$ the cell thickness. This method presupposes conformity of the solution to Beer's law.

An interesting extension of this method to the determination of manganese and chromium in the same sample of steel has been described by Silverthorn and Curtis *(196)*.

h. **Determination of Color Analysis Constants.** For the system selected for measurement determine the transmittancy, if a transparent medium, or the reflectance, if an opaque medium, at intervals of 10 mμ from 400 to 700 mμ and plot the transmittancy (or reflectance)-wavelength curve.

Follow the directions given by Hardy *(83)* in calculating the trichromatic coefficients from this curve. Either the weighted or selected ordinate method may be used. The procedure is simplified if a calculator *(207)* is available. Having determined the trichromatic coefficients, the luminance, dominant wavelength, and purity may then be obtained. A copy of Hardy's charts should be available for this purpose.

Spencer *(199)* has proposed an extension of such calculations to introduce the adaptation of the eye into the color specification system.

i. **Effect of Solution Variables.** A number of factors affect the color of various solutions, either during production of the color or following its development. Spectrophotometric curves provide an excellent means of measuring and studying these effects. An automatic recording instrument is particularly desirable if the system undergoes rapid change.

Space is not available to provide detailed experiments under all the subheadings. Where details are not given, the outline indicates the factor, a solution that will illustrate it, and a reference where details may be found.

a.′ **Sensitivity of Color-Forming Reagents.** Pipet 10-ml. portions of a solution containing 25 mg. of iron per liter into a series of 100-ml. volumetric flasks. Develop colors by means of the reagents in Table X, under conditions specified therein, and determine the spectral transmittancy curves. From the observed transmittancies, at the indicated wavelengths of maximum absorptance, calculate the concentrations, in mg. per liter (p.p.m.), which would give 50 per cent transmittancy at these wavelengths. Arrange the reagents in the order of decreasing sensitivity. An alternative calculation, if the required chemical formulas are known, is the molecular extinction coefficient.

b.′ **Effect of Excess Color-Forming Reagent.** Determine the curves, as suggested in Section "a'," for iron plus ammonium thiocyanate,



ferron, and 1,10-phenanthroline, using the conditions specified in Table X. Then repeat the determinations with two and with four times the amount of reagent used at first. Plot the curves as a transmittancy-wavelength graph.

c.′ **Effect of Time on Development of Color.** To 5 ml. of a solution containing 0.1 mg. of potassium dihydrogen phosphate per ml. add 5 ml. of 5 per cent ammonium molybdate solution (in 2.5 M. sulfuric acid). To this add 1 ml. of 2 per cent hydroquinone and 1 ml. of 20 per cent sodium sulfite and make up to 100 ml. Determine the transmittancy at various time intervals over a period of 48 hours. Plot the curves as before.

d.′ **Effect of Temperature on Development of Color.** To determine the effect of the initial temperature on the rate of development of the color the experiment in the previous section may be repeated. To do this, prepare in the same way three different solutions by using reagents previously brought to 25, 50, and 75°C. before mixing. Determine the transmittancies as quickly as possible. Keep the stock solutions so prepared at the respective temperatures, and at the end of an hour determine the transmittancies again. Plot transmittancy-wavelength curves.

e.′ **Effect of pH on Color.** The effect of pH on color may be studied easily with a solution such as potassium dichromate, in which there is a simple equilibrium, as represented by the equation,

$$Cr_2O_7^{--} + H_2O \rightleftarrows 2HCrO_4^- \rightleftarrows 2H^+ + 2CrO_4^{--}$$

Prepare two series of solutions of potassium dichromate, one M/250 and one M/1000, ranging in pH from 3 to 9 in units. As solvent use standard buffer solutions and adjust the pH finally with dilute sulfuric acid or potassium hydroxide. Determine the transmittancies and plot the curves in the usual way.

Strikingly different effects may be obtained with solutions of iron and the color-forming reagents listed in Table X. For this purpose use ferron, salicylaldoxime, and 1,10-phenanthroline or 2,2′-bipyridyl. After the reactants are mixed and diluted nearly to 100 ml., add dilute hydrochloric acid or sodium hydroxide to adjust the pH to the desired value (determined preferably with a glass electrode), and then bring the volume to 100 ml. Determine the curves, at intervals of about 1 pH unit, from 1 to 5 for ferron, and from 3 to 9 for salicylaldoxime and for 1,10-phenanthroline or 2,2′-bipyridyl.

Interest may be added by determining the family of curves showing the hues of an acid-base indicator at pH values through its transformation range *(56)*. Universal indicators show a greater range of hues *(237)*.

TABLE X.

*Reagents for Iron*

| Compound | Concn. % | Vol. Ml. | Acidity Adjustment | pH | Max. Absptn. Mμ | Reference: Anal. Ed., Ind. Eng. Chem. |
|---|---|---|---|---|---|---|
| Ammonium thiocyanate*..... | 20 | 2 | $HNO_3$, 5 ml., 6 M. | 0.1-1.2 | 470 | 13, 551 (1941) |
| Ferron..... | 0.04 | 10 | $NaC_2H_3O_2$, HCl | 2.8-3.2 | 440 | 9, 406 (1937) |
| Sodium salicylate..... | 10 | 1 | $NH_4OH$ and $CH_3COOH$ | 2.6-2.8 | 520 | 10, 136 (1938) |
| Salicylaldoxime..... | 0.1 | 10 | 1 gm. $NH_4C_2H_3O_2$ | 6.5-7.5 | 490 | 12, 448 (1940) |
| Mercaptoacetic acid..... | 10 | 2 | $NH_4OH$, 10 ml., 3 M. | 10 | 535 | 10, 7 (1938) |
| Kojic acid..... | 0.1 | 10 | $NH_4C_2H_3O_2$, 10 ml., 1 M. | 5.5-6.5 | 440 | 13, 612 (1941) |
| 1,10-Phenanthroline**..... | 0.1 | 5 | ..... | 2-9 | 508 | 10, 60 (1938) |
| 2,2'-Bipyridyl**..... | 0.5 | 5 | ..... | 3-9 | 522 | 14, 364 (1942) |

* Add 60 ml. of acetone before the reagent.
** Reduce the iron with 1 ml. of 10 per cent hydroxylamine.

f.′ **Stability of Color.** Use the iron solution and ammonium thiocyanate specified in Table X. Omit the acetone and use hydrochloric acid to bring the pH in the range 1.5 to 2.5. Determine the curve immediately and at intervals of several hours.

g.′ **Effect of Solvent.** To 10 ml. of a solution containing 0.04 mg. of iron per ml. add 5 ml. of 6 M. nitric acid, 2 ml. of 20 per cent ammonium thiocyanate, dilute to 100 ml., and determine the transmittancy curve.

Repeat this determination using enough acetone for dilution in order to make its final concentration 20, 40, 60, and 80 per cent by volume.

h.′ **Effect of Diverse Ions.** In many cases the presence of ions other than the one to be determined colorimetrically interferes more or less seriously with the color. The magnitude of the effect may be calculated from the relationship,

$$T_1 = T_2 C_1 / C_2$$

$T_1$ and $C_1$ and $T_2$ and $C_2$ being, respectively, the transmittancies, at a given wavelength, and the concentrations of the unknown and standard solutions.

Instead of comparing the transmittancy of the unknown solution with that of a standard, the change in transmittancy produced by adding a known amount of the desired constituent to the unknown may be determined. The unknown concentration, $x$, is then calculated according to Beer's law as follows *(157)*:

$$T_x = T^{\frac{x}{a+x}}_{a+x}$$

$$x = \frac{a \log_{10} T_x}{\log_{10} \frac{T_{a+x}}{T_x}}$$

in which $T_x$ is the transmittancy of an aliquot of the sample and $T_{a+x}$ is the transmittancy of a similar aliquot containing a small additional quantity of the desired constituent, $a$. Uncertainties arising from the presence of other constituents, turbidity, extraneous color, or other sources, except presence of the desired constituent in the reagents, are thus compensated.

In the references in Table X, or in the sections mentioned, examples of the following types of interference may be found:

a.″ **Increase in Color.** Both the interfering ion and the ion to be determined form similarly colored systems with the reagent (arsenate

and silicate ions in the molybdenum blue method for phosphorus—see Section "c'").

b." **Decrease in Color.** The interfering ion forms a colorless complex with the color-forming reagent whose effective concentration is thus reduced (ferric ion + aluminum ion + ferron); the interfering ion reacts with the ion to be determined by forming a colorless complex (ferric ion + pyrophosphate ion + ferron), or by rendering the ion to be determined inactive through oxidation or reduction (ferric ion + chlorostannous ion + thiocyanate ion); the interfering ion forms a precipitate which reduces the transmittancy (ferric ion + tungstate ion + thiocyanate ion).

c." **Change of Hue.** The interfering ion and the reagent form a colored complex having a hue different from that formed with the desired constituent (ferric ion + molybdate ion + salicylaldoxime); the interfering ion is itself colored (ferric ion + cobaltous ion + thiocyanate ion).

j. **Spectral Centroid.** The product of the transmittance (reflectance), at any given wavelength, and the luminosity at the same wavelength gives the luminosity of the sample for this wavelength. A curve coordinating these values (calculated for wavelengths 410, 410, .... 690, 700 m$\mu$) with wavelength gives the luminosity curve for the sample *(53)*. The centroid of the area included between this curve and the wavelength axis is called the spectral, or wavelength, centroid, or the spectral center of gravity of the sample. Priest noted that "if any two lights, however, different in spectral distribution, excite colors of the same quality, the wavelengths of the centroids of their spectral distribution are coincident" *(171)*. Therefore, all colors that give the same sensation have the same spectral centroid *(53)*. There is mentioned in the report of the Colorimetry Committee *(34d)* the significance of this quantity in specifying filters.

To calculate the centroid, each wavelength used is multiplied by the luminosity value for that wavelength. The sum of these values for all the wavelengths used is then divided by the sum of the luminosity values. Or, in terms of the report of the Colorimetry Committee (*34d*, p. 674)

$$\lambda = \int_0^\infty \lambda \cdot T_\lambda \cdot P_\lambda \cdot \bar{y}_\lambda \cdot d\lambda \,/\, \int_0^\infty T_\lambda \cdot P_\lambda \cdot \bar{y}_\lambda \cdot d\lambda$$

in which, for each wavelength used, $T$ = the transmittance, $P$ = the spectral distribution of the incident light (for illuminant $C$, see Fig. 47), and $\bar{y}$ = the luminosity data for the human eye (see curve $\bar{y}$, Fig. 48).



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