

COLORIMETRIC METHODS OF ANALYSIS

*Including Some Turbidimetric and
Nephelometric Methods*

By

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PREFACE TO THE THIRD EDITION

BECAUSE of the tremendous volume of material published on colorimetric methods—and particularly on photometric methods—during the ten years which have elapsed since publication of the second edition, the size and scope of the present edition have necessarily been increased. Nephelometric and turbidimetric methods—minor in number but still important—are included.

As in the second edition, the aim has been completeness, but even so, much condensation was found necessary. Many of the older references given in the second edition have been deleted; policy has been to cite the majority of new references. Description of new equipment designs is necessarily limited to the commercially more important types. Some unusual modifications are outlined briefly. Expansion of text necessitated a change from two to three volumes, covering in Volume I Theory, Instruments, pH; Volume II Inorganic; and Volume III Organic Applications.

Alternative methods are still given, since that which is satisfactory for one purpose may be unsuited to another. Because the preparation of different types of samples is often the most difficult and troublesome part of a determination, much space has been devoted to this.

In this revision, in addition to study of the extensive journal literature, careful attention has been given to various related volumes which have appeared, particularly *Organic Reagents in Inorganic Analysis* by Ibert Mellan (1941); *Kolorimetrische Analyse* by Bruno Lange (1941); *Photometric Clinical Chemistry* by William S. Hoffman (1941); *Optical Methods of Chemical Analysis* by T. R. P. Gibb, Jr. (1942); *Colorimetric Determination of Traces of Metals* by E. B. Sandell (1944); *Metallurgical Analysis by Means of the Spekker Absorptiometer* by F. W. Haywood and A. A. R. Wood (1944); *Colorimetry for Chemists* by M. G. Mellon (1945); *Colorimetric Analysis* by Noel L. Allport (1945); and others.

PREFACE TO THE THIRD EDITION

Reviews of the previous edition were studied for guidance in meeting constructive criticisms. Also many helpful letters from readers relating experiences with specific methods were given due consideration; our gratitude is expressed here to these people.

The authors wish to express appreciation to Leonard C. Cartwright for help with Chapters 2 and 19, to Sally Cohen for careful editorial aid, and to Anne Burdé for painstaking preparation of manuscript.

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CHAPTER I

COLORIMETRIC METHODS

A COLORIMETRIC method as applied to chemical analysis fundamentally consists of treating a solution of a substance with a reagent in such a way as to produce a color which is proportional in intensity to the amount of the substance present in the solution. It is desirable but not essential that the proportionality be linear. The methods are applicable to the determination of many metals, radicals and organic compounds, often after elimination of interfering ions or radicals. The unknown is spoken of in this general discussion as the test substance. After the color has been produced, the solution containing an unknown amount of test substance is compared with a standard solution by one of five methods and the result read either by eye or photoelectrically. The older methods involve a simple comparison of sample versus standard, but modern technics tend to measure the color of the sample in absolute terms and compare with predetermined numerical absorption standards. Whichever method is applied gives a comparison by color as distinguished from the physical analysis of color.

Measurement. The intensity of color of a sample may be read by the eye, by a photoelectric cell, by a thermopile or by the record on a photographic plate, the last two being seldom used. The instrument used is not the color meter implied in the term "colorimeter," since only in the spectrophotometer is intensity of color measured quantitatively. That instrument as applied to colorimetry records the absorption of a given band of wave lengths of light.

Colorimetric methods for at least iron and cobalt are more than a century old. The importance of colorimetry has been promoted by rapid improvement in recent years in apparatus for measurement of the absorption of light by aqueous solutions.¹ That improvement has been quite largely in photoelectric methods which has in turn led to the development of superior methods of test. This is indicated by the trend of several ASTM committees to change to photoelectric methods.² Accur-

¹ G. E. F. Lundell, *Proc. Am. Soc. for Testing Materials*, 44, 709 (1944).

² J. J. Stumm, *Ibid.*, 44, 749-53 (1944); V. A. Stenger, *Ibid.*, 754-61; C. Zischkau, *Ibid.*, 762-8; Arba Thomas, *Ibid.*, 769-78.

acy of visual methods ranges downward from 5 per cent and rarely exceeds 2 per cent. Photoelectric methods are often reproducible to 0.2 per cent but that does not mean necessarily that degree of precision is obtainable. Whether such is the case depends on other factors in the determination, prior to the instrumental reading.

The methods of colorimetric chemical analysis do not include physical color analysis. Thus, the term "colorimetric analysis," as used by the chemist and the physicist, has a different meaning. Chemical methods are applicable to a reasonably limited number of materials; physical methods analyze the wave-length distribution of any transparent or opaque colored substance. Colorimetric chemical analysis finds a matched transmittance by test solution and standard, sometimes indirectly through a comparison curve; colorimetric physical analysis reports quantitatively the transmittance or reflectance. To integrate related optical methods as applied in chemistry, photometric analysis in the general use of the term is measurement of the intensity of transmittance of the entire spectrum; spectrophotometric analysis is measurement of the same factor as applied to limited wave lengths, usually narrow bands; abridged spectrophotometry differs from spectrophotometric analysis only in that the wave band is less accurately restricted. Abridged spectrophotometry and spectrophotometric analysis are applied in colorimetric analysis for measurement of absorption or the reciprocal transmittance.

Series of Standards. The sample, diluted to a definite volume, is compared with a series of standards of the same volume, in which the amount of test substance is known. The value of the unknown is either taken to be that of the standard to which it most nearly conforms or estimated from that standard. In this way the amount of test substance present is obtained without calculation, since, if the volume and color of the unknown and the standard are the same, their contents of test substance will be identical. Beer's law need not apply and dichromatism can be tolerated.

Absorption. This is closely related to the series-of-standards method. The transmittance of a series of developed standards is measured, ordinarily with a restricted wave band, and plotted on a calibration chart. The developed samples are similarly read and the analytical values read from the chart. The chart normally reads either in transmittance, which is a logarithmic curve, or in $-\log_{10}$ of the transmittance known as extinction, which is linear. Beer's law need not apply to the system;

if it does, the transmittance curve will be smooth and the log curve will be a straight line.

Dilution. The standard and sample are placed in graduated tubes of similar diameter, and the darker diluted with the same concentration of reagents as is present in sample and standard. The end point is when the color of one viewed horizontally through the tube after mixing is the same as that of the other. When this point is reached, if all necessary conditions have been observed, each unit volume of one solution must contain the same amount of test substance as each unit volume of the other and the amount in the unknown is to the amount in the known directly as their volumes. Beer's law need not hold for the system.

Balancing. A sample solution is placed in a flat-bottom graduated tube and a standard solution added to a similar tube, until the color intensities, when observed through the lengths of the columns of liquid, are identical. The amount of test substance in each tube will then be the same, and, since the quantity per unit volume in the standard is known, the total amount in the standard may be calculated. This is identical with the quantity in the sample. If the tubes are not of the same cross section, the amount of test substance per unit of cross section is the same, and the concentrations are to each other inversely as the depths of solution. More briefly, their concentrations are inversely proportional to their depths. This is the method employed with many commercial instruments. Beer's law must hold for the system.

Duplication. The sample is made up to a definite volume, and nearly that volume of water in a similar container is treated with the same reagents for bringing out the color of the solution, as were used with the sample. A concentrated solution of standard is added to the blank from a buret, drop by drop when the end point is near. The volume of the blank is then brought up by addition of more water until the two colors and volumes are identical. The amount of standard used in making the duplicate is a measure of the amount of test substance in the sample. Because of its resemblance to usual titration methods this is sometimes called colorimetric titration. Beer's law need not hold for the system but reaction must be practically instantaneous.

Extent of Use. The significance of chemical colorimetry is indicated by a conservative estimate of about 25,000 colorimeters in use in 1939,³

³ Ralph H. Müller, *Ind. Eng. Chem., Anal. Ed.*, **11**, 1-17 (1939).

a figure which must have been multiplied by a large whole number since the estimate was made. This cannot include the many laboratories where sets of Nessler tubes or test tubes are used for occasional determinations.

Colorimetric methods often give results in 5 minutes to 1 hour from the time the determination is begun, which is in many cases a small fraction of the time in which similar determinations could be made by other methods. It may be said for colorimetry in general that its methods are rapid and reasonably accurate.

A broad field of usefulness of colorimetry is the determination of impurities in substances easily soluble in water, alkali, or acid. The methods are very sensitive, usually determining 0.5-10 ppm., and on rare occasions detecting one part per billion. In many of its applications, chemical colorimetry is a part of microanalysis.⁴ When applicable the methods are usually simpler, more convenient, and at least as accurate as alternative methods. As a matter of correlation between the various methods the quantities are referred to in fractions of a milligram rather than in γ , micrograms, the 0.001 mg. unit in which microchemistry is often expressed. In general, in giving weights, one more numeral than is really significant has often been cited as a guide to the analyst.

The methods are seldom used to determine amounts much greater than one per cent. In control work where limited accuracy is acceptable they are more widely applicable for determination of major constituents. For example, determination of copper up to 22 per cent in ores with an accuracy of 0.1 per cent has been described.⁵ By use of monochromatic light as many as four substances have been determined in the same solution⁶ and, by proper calculation, overlapping bands can be determined.⁷ The importance of colorimetric methods is further indicated by the periodic appearance of reviews of new instruments and their applications.⁸

⁴ Albert E. Sobel, *Ind. Eng. Chem., Anal. Ed.*, **17**, 242-5 (1945).

⁵ J. P. Mehlig, *Ind. Eng. Chem., Anal. Ed.*, **7**, 387-9 (1935).

⁶ Fritz Weigert, *Ber.* **49**, 1496-1532 (1916).

⁷ Harold W. Knudson, Villiers W. Meloche, and Chancey Juday, *Ind. Eng. Chem., Anal. Ed.*, **12**, 715-19 (1940).

⁸ Ralph H. Müller, *Ind. Eng. Chem., Anal. Ed.*, **7**, 223-6 (1935); *Ibid.*, **12**, 571-630 (1940); *Ibid.*, **13**, 667-754 (1941); S. A. Thiel, *Ber.*, **66B**, 1015-23 (1935); K. S. Gibson, *Instruments*, **9**, 309, 335 (1936); *J. Soc. Motion Picture Engrs.*, **28**, 388 (1937); P. Krumholz, *Scientia Pharm.*, **7**, 103-6 (1936); M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.*, **9**, 51-6 (1937); *Ibid.*, **11**, 80-5 (1939); N. Strafford, *Chem. Soc. Annual Repts.*, **33**, 456-65 (1936); G. Kortüm, *Angew. Chem.*, **50**, 193-204

Development of a Colorimetric Method. In general, in the development of a new colorimetric procedure, only the method by a series of standards can be assumed to be applicable, the standards containing the same amounts of the same reagents as the unknown and prepared at the same time. Absorption may be measured as a method of recording the intensity of the series of standards and as previously indicated will show whether the system conforms to Beer's law. Even if Beer's law is not found to be applicable to the reaction, the dilution method may usually be applied within limits. If Beer's law holds, the method of balancing is applicable within the limits in which the law has been found to apply. If the color is permanent and develops nearly instantaneously, the method of duplication is applicable.

If the color develops over a wide range of wave bands, then the eye with reasonable acuity over the entire range will respond to the intensity with greater accuracy than an instrument reading a restricted wave band. If, on the other hand, the color change developed by the reagent lies almost entirely in a restricted wave band, the use of filter methods will be definitely preferred. The first condition corresponds with a dull and nonspecific color or, in the extreme case, with a gray; the latter with a bright color.

Frequently, the product obtained from the test substance precipitates above a definite concentration, in which case that sets a limit. The lower limit is usually that at which the color is just perceptible but the absolute accuracy becomes less as this lower limit is approached. In many cases, before precipitation is reached, the substance becomes colloidal rather than dissolved, and sometimes, but not always, the methods become nephelometric under these conditions. The formation of particles large enough to make the method nephelometric can sometimes be avoided by addition of a protective colloid, such as a water-dispersible gum.

(1937); Edwardo Coffari, *Chim. Industria*, (Italy) 19, 255-6 (1937); S. E. Q. Ashley, *Ind. Eng. Chem., Anal. Ed.*, 11, 72-9 (1939); W. D. Wright, *Repts. Progress Physics*, 7, 36-40 (1940); Manfred Richter, *Arch. tech. Messen.*, No. 111, T99-100, No. 113, T123-4 (1940); G. Kortüm and J. Grambow, *Angew. Chem.*, 53, 183-7 (1940); O. H. Weber, *Ibid.*, 54, 56-7 (1941); D. L. Tilleard, *J. Oil Colour Chem. Assoc.*, 25, 227-39 (1942); R. P. MacFate, *Am. J. Clin. Path., Tech. Sect.*, 7, 55-65 (1943); W. Kluge et al, *Die Chemie*, 55, 362-6 (1942); 56, 183-4 (1943); Maria I. Ardao, *Ph.*, 1943, No. 2, 11-14, No. 3-4, 28-30, No. 5, 3-6; A. L. Davydov, *Trudy Vsesoyuz. Konferentsii Anal. Khim.*, 2, 233-51 (1943); J. S. Fawcett, *Proc. Phys. Soc.*, (London) 56, 8-21 (1944); E. I. Stearns, *Am. Dyestuff Repr.*, 33, 1-6 (1944); M. G. Mellon, *Proc. Am. Soc. for Testing Materials*, 44, 735-9 (1945).