

Reprinted from *ANALYTICAL CHEMISTRY*, Vol. 33, Page 1715, November 1961

Absorptivity Correction in Multicomponent Spectrophotometric Analysis

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► A procedure for correction of absorptivities in the spectrophotometric analyses of mixtures is described wherein the absorptivities corresponding to the concentration of each component in a mixture are corrected by the ratio of observed absorbance of the mixture to the sum of absorbances of the separate components. Very accurate determinations of concentrations are obtained when the correction is used. Its application to the infrared

analysis of a ternary mixture of phenols and to a two-component dye mixture in the visible range is presented.

SPECTROPHOTOMETRIC analysis of mixtures of absorbing substances is achieved by the solution of simultaneous linear equations based upon exact absorptivity values. In practice this requirement is fulfilled with difficulty because of the frequency of deviations

from Beer's law. The absorbance-concentration relationship of individual components may be nonlinear, or the absorbances of the components may be nonadditive. Deviations may be concealed within the limits of experimental variation while individual and composite linearity are assumed to be present.

This report describes a procedure whereby correction for nonadditivity is applied in conjunction with absorb-

ance-concentration curves of individual components, giving absorptivity values applicable to a particular mixture and accurate determinations of concentration. The procedure is referred to herein as absorbance ratio correction, the ratio being that of observed absorbance of a mixture to the sum of absorbances of the separate components. When the individual absorptivities corresponding to the concentration of each component in a mixture are corrected by this absorbance ratio, precise absorptivity values applicable to the mixture are obtained. The correction has been employed in this laboratory in the infrared analysis of a ternary mixture of phenols. It has also been applied for illustrative purposes to a two-component dye mixture in the visible light range.

THEORETICAL

The pertinent absorption law relationships are summarized as follows:

$$\log \frac{I_0}{I} = abc = A$$

where

- I_0 = radiant energy transmitted through cell containing solvent
- I = radiant energy transmitted through cell containing absorbing substance
- a = absorptivity, 1000 sq. cm. per gram
- b = cell path, cm.
- c = concentration, grams per liter
- A = absorbance

For solutions containing more than one absorbing substance and assuming conformity to the absorption law

$$A_{\text{observed}} = A_1 + A_2 \dots + A_n = A_{\text{theoretical}} \\ = b(a_1c_1 + a_2c_2 \dots + a_nc_n)$$

With a cell path of 1 cm., b is omitted. When all the absorptivities are known, A_{obsd} is measured at n wave lengths and n equations are solved to obtain the n concentrations.

The absorptivities correspond to the slope of a line connecting a point on the individual absorbance-concentration curves with the origin. If the slopes are not constant, individual nonlinearity is said to be present. Even in the absence of individual nonlinearity, A_{obsd} may not be equal to A_{theo} and the condition is referred to as nonlinearity of total concentration or nonadditivity.

In the case of nonadditivity, let a proportionality term be defined as

$$\frac{A_{\text{obsd}}}{A_{\text{theo}}} = r$$

The assumption is made that

$$A_{\text{obsd}} = ra_1c_1 + ra_2c_2 \dots + ra_nc_n$$

At a given wave length, r is the same for all components and will be more or less than 1 according to whether the deviation is positive or negative. At

each of n wave lengths, a different r is applied according to the absorbance ratio at that wave length. Thus the corrected equations take the form

$$A'_{\text{obsd}} = r'a_1c_1 + r'a_2c_2 \dots r'a_nc_n$$

$$A''_{\text{obsd}} = r''a_1c_1 + r''a_2c_2 \dots r''a_nc_n$$

$$A^n_{\text{obsd}} = r^na_1c_1 + r^na_2c_2 \dots r^na_nc_n$$

METHODS OF CORRECTION USED IN MULTICOMPONENT ANALYSIS

Deviations from the absorption law are more frequently encountered in multicomponent analysis than in analysis of single substances. They may be chemical or physical in nature, because of interaction of the absorbing entities with each other or with the solvent. Instrumental factors may be responsible, such as poor resolution or stray light which contributes to lack of monochromaticity in the incident radiation.

If experimental conditions cannot be altered to eliminate the deviations (dilution, change of solvent, smaller slits, etc.), some type of correction must be applied. The one most widely used is empirical correction for nonlinearity of individual components (2, 4).

Graphical correction to offset non-additive effects is described by Stearns (6), using the same dye mixture to which the absorbance ratio correction is applied in this study. The ratio of calculated total concentration to true total concentration is plotted against concentration of one of the components. This curve is used in conjunction with a second curve depicting calculated *vs.* true concentration of the same component to arrive at the correct analysis. Vaughn and Stearn (7) used differences in absorbance at different wave lengths to prepare a three-component graph for isomeric xylene mixtures. Either procedure corrects for nonadditive absorbances. In each case, however, the prepared graphs apply to a single total concentration, and if the concentration range of interest is large, a family of such graphs may be required. This limitation applies to all correction curves of the type relating true to calculated concentration, if absorbances are not additive.

Another method of correction for nonadditive effects, also presented by Stearns (6), is called "incremental analysis." The absorptivities are obtained by the separate addition of each component to the unknown mixture or to a standard mixture of similar composition. The change in absorbance divided by the increase in concentration gives the absorptivity prevailing in the mixture. Choice of the incremental concentration involves a compromise, since it must be large enough to provide an accurate measure of change in absorbance, yet small enough to cover a range where the absorptivity is essentially constant. Beroza (1)

has used this procedure in conjunction with the differential technique.

Correction procedures other than the empirical and incremental types described above have not been noted in a review of the literature. The absorbance ratio correction was devised when difficulty due to nonadditive absorbances was experienced in the infrared analysis of a general-purpose disinfectant formulated from the sodium salts of *o*-phenylphenol, 4-chloro-2-phenylphenol, and 6-chloro-2-phenylphenol.

EXPERIMENTAL

Sample Preparation. A 10.000-gram sample of the disinfectant was dissolved in 25 ml. of distilled water in a separatory funnel and shaken with 20 ml. of 5*N* H₂SO₄. The converted phenols were extracted with 10-, 5-, and 5-ml. portions of CS₂, collected in a 25-ml. volumetric flask, and adjusted to volume with CS₂ at a temperature of 22° to 24° C. The extract was transferred to a stoppered Erlenmeyer flask containing 5 grams of Na₂SO₄, shaken until clear of water, then filtered through glass wool into another stoppered flask for retention. Transfers were made quickly to avoid loss of solvent.

Instrumental Measurement. A Beckman IR2 spectrophotometer with sodium chloride optics and cells was used for the analysis. Absorbance measurements were made by null point readings. The wave lengths used were 8.78 microns for the 6-Cl-2-*φ*-phenol, either 9.04 or 9.90 microns for the *o*-*φ*-phenol, and 11.33 microns for the 4-Cl-2-*φ*-phenol. Corresponding slit settings were 0.55, 0.60, 0.72, and 1.07 mm., respectively. A constant cell path of 0.105 mm. was used for all samples and standards, and readings were made against a 0.106-mm. reference cell of CS₂. Temperature was controlled between 22° and 24° C. All solutions were read in duplicate and multiple readings were taken on each cell filling when instrument instability was present. All readings were corrected for the absorbance of the sample cell filled with CS₂, which was read before and after each series of measurements.

Standard solutions of the phenols in CS₂ covering the ranges of concentration to be encountered in the samples were read at each wave length and used to prepare the individual standard (absorbance *vs.* concentration) curves. The purified standards were laboratory-prepared with melting points of 57.2-8.5°, 36.5-7.4°, and 74.8-5.3° C., for *o*-*φ*-phenol, 4-Cl-2-*φ*-phenol, and 6-Cl-2-*φ*-phenol, respectively. A synthetic mixture approximating the composition of the samples was prepared and the absorbances of synthetic mixture and samples were read at the selected wave lengths.

Data Treatment. Absorptivity values were computed by dividing absorbance by corresponding concentration at regular intervals along each

of the individual standard curves. The values in this case were tabulated. However, if absorptivity is changing rapidly with concentration, a plot of absorptivity vs. concentration would be preferable.

The absorbances corresponding to the concentration of each component in the synthetic mixture were read from the individual standard curves and summed to obtain the theoretical absorbance. The absorptivity values corresponding to the concentration of each component were read from the tables. The absorptivities were corrected by the absorbance ratios, $\frac{A_{\text{obsd}}}{A_{\text{theo}}}$ of the synthetic mixture at the appropriate wave lengths, and the corrected absorptivities were used to calculate the sample concentrations. For calculation the method of Crout (3, 5) was preferred when absorptivities and equations were changing frequently.

A second synthetic mixture was then prepared corresponding to the calculated sample concentrations. If the calculated concentrations were correct, the absorbance readings of this second mixture matched those of the unknown sample. If a match of absorbance readings was not obtained, new corrected absorptivities were similarly derived from the second synthetic mixture—that is, absorbances and absorptivities corresponding to the concentration of each component in the second mixture were read from the graphs and tables. The theoretical absorbance was obtained from the sum of the individual absorbances. The absorptivities were multiplied by the ratio $\frac{A_{\text{obsd}}}{A_{\text{theo}}}$ and a second calculation was made with these new corrected absorptivities.

The second calculation was in turn tested by preparation of a third synthetic mixture, and new corrected absorptivities were derived, if necessary, from the third standard. By this process of successive approximation the correct analysis could be ultimately obtained, even though the initial synthetic mixture used for calculation differed greatly from the unknown sample. The number of approximations required in a particular case would depend upon the degree of nonadditivity present, or, more precisely, upon the rate of change of nonadditivity with change in concentration. This determines the amount of similarity necessary between the standard mixtures and the samples.

Analysis of Synthetic Dye Mixtures.

It was of interest to study the effect of absorbance ratio correction on a grossly nonadditive mixture in a different spectral range. For this purpose standard solutions and mixtures of Direct Sky Blue 6B Ex (Ciba) and Chrysophenine G (Arnold Hoff-

man) in water solution were prepared and read in a Beckman DU spectrophotometer with 1-cm. Corex cells. Absorbance readings were made at 490 μ for the yellow and 520 for the blue, since a relatively high total concentration of 50 mg. per liter, selected to exaggerate deviations, precluded the use of absorption peaks. Data treat-

ment was similar to that used for the phenol analyses.

RESULTS

Recoveries from synthetic phenol mixtures are listed in Table I. The maximum relative error is 1%, with an over-all standard deviation of $\pm 0.42\%$

Table I. Analysis of Synthetic Phenol Mixtures with Absorbance Ratio Correction

Synthetic Mixture	o-Phenylphenol			4-Cl-2-phenylphenol			6-Cl-2-phenylphenol			
	Known, g./ml.	Calcd., g./ml.	Error, %	Known, g./ml.	Calcd., g./ml.	Error, %	Known, g./ml.	Calcd., g./ml.	Error, %	
A	0.1018	0.1018	0	0.1451	0.1462	+0.8	0.0493	0.0498	+1.0	
B	0.0976	0.0975	-0.1	0.1429	0.1431	+0.1	0.0485	0.0485	0	
C	0.0996	0.0986	-1.0	0.1469	0.1469	0	0.0537	0.0538	+0.2	
D	0.0989	0.0993	+0.4	0.1486	0.1483	-0.2	0.0557	0.0555	-0.4	
E	0.0799	0.0803	+0.5	0.1621	0.1624	+0.2	0.0639	0.0638	-0.2	
F	0.0809	0.0811	+0.2	0.1631	0.1631	0	0.0651	0.0649	-0.3	
G	0.0645	0.0647	+0.3	0.1373	0.1372	-0.1	0.0500	0.0500	0	
Standard deviation			± 0.51				± 0.33	± 0.47		

Table II. Data Sheet for Correcting Absorptivities

	Wave Length, Microns				
	8.78	9.04	11.33		
A_{obsd}	0.507	0.394	0.443		
A_o	0.0856	0.1415	0.0185		
A_4	0.1490	0.1900	0.4216		
A_6	0.2590	0.0310	0.0108		
A_{theo}	0.4936	0.3625	0.4509		
$\frac{A_{\text{obsd}}}{A_{\text{theo}}}$	1.027	1.087	0.982		
Uncorrected absorptivities					
a_o	1.07	1.77	0.232		
a_4	0.919	1.17	2.60		
a_6	4.05	0.485	0.169		
Corrected absorptivities					
a_o	1.10	1.92	0.228		
a_4	0.944	1.27	2.55		
a_6	4.16	0.527	0.166		
	Known Concn., G./Ml.	Concn. Calcd. from Uncorrected Absorptivities, G./Ml.	% Error	Concn. Calcd. from Corrected Absorptivities, G./Ml.	% Error
C_o	0.0799	0.1015	+27.0	0.0803	+0.5
C_4	0.1621	0.1573	-3.0	0.1624	+0.2
C_6	0.0639	0.0627	-1.9	0.0638	-0.2

Subscript o = o-phenylphenol.
Subscript 4 = 4-Cl-2-phenylphenol.
Subscript 6 = 6-Cl-2-phenylphenol.

Table III. Comparison of Recovery with Two Wave Lengths for o-Phenylphenol

	Known Concn., G./Ml.	Calcd. Concn. ($\lambda = 9.04 \mu$), G./Ml.	Relative Error, %	Calcd. Concn. ($\lambda = 9.90 \mu$), G./Ml.	Relative Error, %
C_o	0.1000	0.0998	-0.3	0.1000	0
C_4	0.1500	0.1497	-0.2	0.1497	-0.2
C_6	0.0500	0.0501	+0.2	0.0501	+0.2

Table IV. Reproducibility of Replicate Analyses

	Analysis, %			Mean
	1	2	3	
	Sample 1			
<i>o</i> -Phenylphenol	28.54	28.63	28.70	28.6
4-Cl-2-phenylphenol	40.23	40.21	40.40	40.3
6-Cl-2-phenylphenol	14.23	14.29	14.33	14.3
Total phenol	83.0	83.1	83.4	83.2
Water				16.7
Total				99.9
	Sample 2			
<i>o</i> -Phenylphenol	28.80	28.73	28.96	28.8
4-Cl-2-phenylphenol	40.32	40.31	40.30	40.3
6-Cl-2-phenylphenol	14.61	14.58	14.53	14.6
Total phenol	83.7	83.6	83.8	83.7
Water				16.0
Total				99.7

Table II lists the absorbance and absorptivity values of synthetic mixture E of Table I and the recoveries obtained with and without correction. The same order of recovery was obtained for all the mixtures, when uncorrected absorptivities were used for calculation. The serious error in *o*- ϕ -phenol concentration when correction is not applied reflects interference at 9.04 microns from the other phenols, which are responsible for about one half of the total absorbance at that wave length. Table III compares recoveries using either 9.04 or 9.90 microns for *o*- ϕ -phenol, the latter having minimal interference but total absorbance of 0.25 compared to 0.40 to 0.50 for the other wave lengths.

The reproducibility and total recovery for two sample analyses are shown in Table IV. The water analysis was done by toluene distillation and includes a correction for systematic loss, giving 96% recovery.

Synthetic mixtures A, B, C, and D in Table I are representative of the normal composition of the disinfectant. Mixtures E, F, and G are representative

of samples which were sufficiently different in composition that some of the absorbance readings and therefore some of the corrected absorptivities appropriate for calculation differed considerably from those of the normal samples. Mixtures E and F are examples of the use of successive approximation. Mixture E was prepared to correspond to a first calculation of a sample with corrected absorptivities applicable to the normal composition. Mixture F was prepared from the calculation of the same sample with absorptivities derived from mixture E. Although the difference between the two calculations is small, the absorbance readings of F converged upon those of the sample, while the absorbance readings of E did not. (Absorbance readings within ± 0.002 were accepted as a match.)

The recoveries from the synthetic dye mixtures are listed in Table V. The uncorrected recoveries indicate the futility of attempting analysis of mixtures with large composite deviation unless some correction is applied. The individual standard curves from 0 to 50 mg. per liter followed Beer's law or were only very slightly curved. Successive approximation was used to analyze a mixture of 90% yellow and 10% blue starting with absorptivities and absorbance ratios corresponding to a 50%-50% mixture. Three synthetic mixtures were required to arrive at the correct absorptivities and absorbance readings. This range was considered to be a severe test for the dye system because the absorbance ratio at 490 $m\mu$ decreases from 1.5 for the 50%-50% mixture to 1.1 for the 90%-10% mixture.

DISCUSSION

The applicability of absorbance ratio correction to systems other than the two studied here is not known, but the

following empirical interpretation is offered.

The concept of a "change" in absorptivity may be misleading, since it is possible that deviations from Beer's law do not really exist, but rather that situations exist where the law cannot be correctly applied. For example, if nonadditivity occurs as a result of chemical interaction, the product or products of the interaction process are entities with different absorption characteristics, or even no absorption, and these substances must be accounted for in the absorption equation. At the same time the initial concentrations of the interacting components have been altered.

For purposes of analysis, however, the absorptivities may be assigned any appropriate values which will produce correct initial concentrations of the components, and it does not matter whether the change is in absorptivity or concentration or both. The absorbance ratio correction distributes the deviation in observed absorbance proportionately, and perhaps artificially, over all the components, but the validity of this step is ensured by similar treatment of the standard mixture from which the ratio is derived.

If the correction technique does have wider applicability, it can eliminate the necessity for a large number of correction curves, especially in cases where the composition and/or total concentration varies appreciably. Where many samples of similar composition are to be analyzed, absorptivities will be the same for all, and once established may be used without further preparation of synthetic mixtures except as an instrument check. Standard curves need not be prepared for an isolated analysis where the approximate composition is known, since a solution of each component and a mixture containing exactly the same concentrations provide the data for calculation.

It is hoped that this method will prove of value to other laboratories in the analysis of nonadditive mixtures.

LITERATURE CITED

- (1) Beroza, M., *ANAL. CHEM.* 25, 112 (1953).
- (2) Brattain, R. R., Rasmussen, R. S., Cravath, A. M., *J. Appl. Phys.* 14, 418 (1943).
- (3) Crout, P. D., *Trans. Am. Inst. Elec. Engrs.* 60, 1235 (1941).
- (4) Fry, D. L., Nusbaum, R. E., Randall, H. M., *J. Appl. Phys.* 17, 150 (1946).
- (5) Milne, W. E., "Numerical Calculus," pp. 17-25, Princeton University Press, Princeton, N. J., 1949.
- (6) Stearns, E. I., "Analytical Absorption Spectroscopy," M. G. Mellon, ed., pp. 380-5, Wiley, New York, 1950.
- (7) Vaughn, R. T., Stearn, A. E., *ANAL. CHEM.* 21, 1361 (1949).

RECEIVED for review March 22, 1961.
Accepted August 21, 1961.

Table V. Concentration of Synthetic Dye Mixtures

	Known Concn., %	Calculated Concentration, %	
		With ratio correction	Without correction
Blue	20.00	19.99	15.9
Yellow	80.00	80.03	100.3
Blue	40.00	40.01	31.4
Yellow	60.00	59.98	97.9
Blue	60.00	60.01	44.5
Yellow	40.00	40.01	95.6
Blue	80.00	80.00	66.6
Yellow	20.00	20.00	68.4