NEW GRAPHICAL METHODS FOR THE ANALYSIS OF MULTICOMPONENT MIXTURES BY ABSORPTION SPECTROPHOTOMETRY

BY

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A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
(Pharmacy)

at the

UNIVERSITY OF WISCONSIN-MADISON

1979

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Professor Kenneth A. Connors for his guidance and suggestions in this investigation. I am also grateful to Professor Joseph R. Robinson for the use of his digital pH meter.

I am extremely grateful to the University of Benin, Nigeria, for my financial support for this study.

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I. INTRODUCTION

A. Analysis of Mixtures by Absorption Spectrophotometry

Electronic absorption spectrophotometry is the technique most extensively used in official methods as the measurement step in quantitative assay of drugs in dosage forms. This is in part a result of the rapidity, sensitivity and simplicity of the technique. However, these advantages can be lost by the requirement that the actual determinative step be preceded by an isolation procedure because of the absorption of other substances in the system (25). Most of the quantitative analyses utilize the ultra-violet (UV) and visible portion of the electromagnetic spectrum.

Quantitative analysis using UV-visible spectrophotometry relies on Beer-Lambert law. For a single absorbing species, this can be written

$$\text{Log } \frac{I_0}{I} = A = \varepsilon bC \qquad (1)$$

where A is absorbance, I_0 is incident radiant power, I is transmitted radiant power, ε is molar absorptivity, b is internal pathlength of cell and C is molar concentration of absorbing species. Thus, there is a direct proportionality between absorbance and concentration. By simple calibration, on standard solutions, the concentration of an unknown sample can be obtained. Some factors such as variation in

instrumental response, inability of handling the analyte at similar conditions (such as temperature, pressure, etc.) as those of the standard(s), limit the accuracy of this simple method. The standard addition method (5) has been proposed to handle such difficulties and minimize the errors. Also, at high concentrations, because of appreciable absorption, large percentage errors may be introduced in the analysis.

UV-visible spectrophotometry has been extensively used in multicomponent mixture analysis. There are basically three methods of approach. One involves the resolution of the mixture into its components, each of which is then measured by the one-component method. Several separation techniques with UV-visible detection depend on this principle. Another method is useful when the spectra of the components do not overlap; then the one-component method can be applied to each component in turn (1,6-8). Finally, simultaneous determination can be carried out. In some mixtures, 'color' development can help in the overall or selective analysis of the components (8-9) by the simultaneous method.

Basic in the simultaneous method is the individual applicability of the Beer-Lambert law, and additivity of the absorbances of the components. [Methods for correcting for non-ideality have been proposed (1,10-12).] Both conditions are expressed in equation (2)

$$A^j = b \sum_{i,j=1}^{n} \varepsilon_i^j C_i \qquad (2)$$

for a sample containing n components when measurement is made at wavelength j. The conventional method of analyzing the sample is to measure A^j at n wavelengths, and to set up n simultaneous equations, which are used to solve for the n unknown concentrations. In using this procedure, it is required that the wavelengths be properly chosen. Some recommend the wavelengths at which the differences of the absorptivities are maximal (1,13-14), while others recommend wavelengths at which the individual components have maximum absorptivity (15-16). As n increases, the solution of the equations becomes more cumbersome. Also, when one of the components is present in relatively low concentration, large percentage errors are introduced in the analysis (17).

Many schemes have been proposed to simplify the calculations and lessen the amount of time and effort needed to set them up and to use them routinely in analysis. Solution of the equations by computer is invaluable when n is large. The absorbance ratio method (18-24) has been extensively used for the analysis of two-component mixtures and can be used for three-component mixtures (18). This is based on the fact that the ratio of two absorbance values determined on any solution of a single component at two given wavelengths is a constant. For a two component mixture, if one

of the chosen wavelengths is an iso-absorptive point, the plot of absorbance ratio versus relative composition is linear. Non-linear plots result if none of the wavelengths is an iso-absorptive point, but the resulting curve can still be used for analysis. The method of difference spectrophotometry has also been used for mixture analysis if the components can undergo multiple spectral changes (37-38,49). Different types of curve fitting techniques have also been used for mixture analysis. These include: least squares regression fit of calculated results to spectral data (27-28); constrained least squares and simplex optimization (15); and mechanical matching of reference with that of sample (29).

B. Aim of the Research

Pharmaceutical preparations containing two or more ingredients are common, and mixtures occur in many other settings. For example, pharmacokinetics, biopharmaceutics and dosage form stability studies must be developed for a drug and its metabolites or decomposition products. In the synthesis of chemicals and drugs, mixtures result at some stages of the process and these can affect the overall yield. Also in equilibrium studies, mixtures are involved. Thus, multicomponent mixture analysis is important in pharmaceutical research, routine analysis, and quality control; and methods that are fast, simple, precise, accurate,

sensitive and selective are required. The aims of this research are to develop some new spectrophotometric methods for the simultaneous analysis of multicomponent mixtures.

Most simultaneous spectrophotometric methods have been restricted to using absorbance values obtained at a limited number of wavelengths. Since Beer's law is not formulated on such limitations, it should be possible to utilize the entire spectral range within which the particular species absorb radiation. It is one of the aims of this research to show that the entire spectral range can be used for simultaneous multicomponent analysis.

In a second investigation, new graphical techniques will be described for the solution of three- or four-simultaneous equations or as an aid in preparing a reference mixture that will give a spectral match with a given sample mixture.

II. THEORY

A. Graphical Analysis of Two- and Three-Component Mixtures Using the Entire Spectral Range

We propose to use a range of wavelengths rather than just two or three wavelengths, and obtain a graphical solution for the analysis of two- and three-component mixtures. Connors (3), by modifying the so-called "method of proportional equations" (which in principle is similar to the conventional simultaneous equations method of spectrophotometry), obtained a linear graphical method for kinetic analysis of mixtures using many measurement times. By analogy, for a two-component mixture of A and B, if ideal behavior is followed, we can write

$$A_t = \varepsilon_A C_A + \varepsilon_B C_B \qquad (3)^a$$

where A_t, ε_A, and ε_B are, in general, different functions of wavelength, i.e.,

$$A_t = f(\lambda) \qquad (4)$$

$$\varepsilon_A = g(\lambda) \qquad (5)$$

$$\varepsilon_B = h(\lambda) \qquad (6)$$

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[a] In all measurements reported, pathlength of cell is 1 cm.

Equation (3) can also be written in the form

$$\frac{A_t}{\varepsilon_A} = C_A + \frac{\varepsilon_B}{\varepsilon_A} C_B \tag{7}$$

or

$$\frac{f(\lambda)}{g(\lambda)} = C_A + \frac{h(\lambda)}{g(\lambda)} C_B \tag{8}$$

These are the working equations for this type of analysis. The experimental approach is to measure the absorption spectra of A, of B, and of the mixture over a range where both A and B absorb radiation. A_t/ε_A is then plotted vs. $\varepsilon_B/\varepsilon_A$ over this range and the absolute concentrations obtained from the intercept and slope.

Alternatively, equation (3) can be written as

$$\frac{A_t}{\varepsilon_B} = \frac{\varepsilon_A}{\varepsilon_B} C_A + C_B \tag{9}$$

or

$$\frac{f(\lambda)}{h(\lambda)} = \frac{g(\lambda)}{h(\lambda)} C_A + C_B \tag{10}$$

Thus the results of the two plots can be compared as a check and, if there are differences, to determine whether obtaining the concentrations from the intercepts or from the slopes is better.

For a three-component mixture of components A, B and C, we can write

$$A_t = \varepsilon_A C_A + \varepsilon_B C_B + \varepsilon_C C_C \tag{11}$$

If C_t represents the total concentration,

$$C_t = C_A + C_B + C_C \tag{12}$$

hence

$$\frac{A_t}{C_t} = \frac{\varepsilon_A C_A + \varepsilon_B C_B + \varepsilon_C C_C}{C_t} \tag{13}$$

Defining fractional composition as

$$f_A = \frac{C_A}{C_t};\ f_B = \frac{C_B}{C_t} \text{ and } f_C = \frac{C_C}{C_t} \tag{14}$$

where

$$f_A + f_B + f_C = 1 \tag{15}$$

equation (13) becomes

$$\frac{A_t}{C_t} = \varepsilon_A f_A + \varepsilon_B f_B + \varepsilon_C f_C \tag{16}$$

Hence from equation (15)

$$\frac{A_t}{C_t} = (\varepsilon_A - \varepsilon_C)f_A + (\varepsilon_B - \varepsilon_C)f_B + \varepsilon_C \qquad (17)$$

or

$$\frac{\frac{A_t}{C_t} - \varepsilon_C}{\varepsilon_A - \varepsilon_C} = f_A + \frac{\varepsilon_B - \varepsilon_C}{\varepsilon_A - \varepsilon_C} f_B \qquad (18)$$

Thus from a plot of the left hand side of equation (18) versus $(\varepsilon_B - \varepsilon_C)/(\varepsilon_A - \varepsilon_C)$, f_A and f_B can be determined from the intercept at zero and slope respectively, while f_C is obtained from equation (15). Alternatively, $f_A + f_B$ can be obtained from the intercept when $(\varepsilon_B - \varepsilon_C)/(\varepsilon_A - \varepsilon_C) = 1$ and f_B can be determined with knowledge of f_A from the intercept at zero.

C_t is obtained by using measurements at not fewer than two iso-absorptive points. At wavelengths where $\varepsilon_A = \varepsilon_B$, equation (11) can be written as

$$A_t = \varepsilon_A C_A + \varepsilon_A C_B + \varepsilon_C C_C \qquad (19)$$

hence

$$\frac{A_t}{\varepsilon_A} = (C_A + C_B) + \frac{\varepsilon_C}{\varepsilon_A} C_C \qquad (20)$$

Thus from a plot of A_t/ε_A versus $\varepsilon_C/\varepsilon_A$, using values at the iso-absorptive points of components A and B <u>only</u>, C_t is

obtained from the value of A_t/ε_A when $\varepsilon_C/\varepsilon_A = 1$.

Note that the quantity A_t/C_t can be interpreted as an apparent absorptivity.

B. Graphical Solutions for Three- and Four-Component Mixtures Using Triangular and Tetrahedral Plots Respectively

Full-Range Solutions

Connors (4), using a "concentration-time matching" approach to the kinetic analysis of mixtures, developed a graphical kinetic method for the analysis of three-component mixtures. We propose to apply a similar technique for the analysis of multicomponent mixtures spectrophotometrically. The goal of the matching technique is to prepare a reference mixture whose absorption spectrum matches that of the sample to be analyzed. At such a matching condition, the composition of the sample is the same as that of the reference. To do this, the absorption spectrum of the sample is measured. Reference mixtures of the sample substances in known concentrations are prepared and their spectra measured. The reference concentrations are adjusted until an exact match with the sample is obtained.

If the analytical goal is a match in which each reference concentration, expressed as a percentage of the total, is within 5% (absolute) of the corresponding sample percentage, each component concentration (with this

criterion) is capable of existing in one of twenty possible levels. If the total reference concentration is set equal to the total sample concentration, then there are $20^{(n-1)}$ possible different reference solutions, only one of which is identical with the sample solution. This will require much effort. The practical problem is to obtain good matching without preparing too many solutions.

To do this, a graphical method is used. For a mixture of three components A, B and C, any solution of A, B, C can be represented by a point in a triangular composition diagram. In such a diagram, each vertex represents a pure component, while the three sides represent the possible binary mixtures. The side opposite the vertex represents zero fraction of that substance and the fractional composition increases with distance from the opposite side to the vertex. Generally, for a three-component mixture, we can write

$$A^1 = \varepsilon_A^1 C_A + \varepsilon_B^1 C_B + \varepsilon_C^1 C_C \tag{21}$$

$$A^2 = \varepsilon_A^2 C_A + \varepsilon_B^2 C_B + \varepsilon_C^2 C_C \tag{22}$$

$$A^3 = \varepsilon_A^3 C_A + \varepsilon_B^3 C_B + \varepsilon_C^3 C_C$$

where A^1, A^2 and A^3 are absorbances at three different wavelengths. Dividing by total concentration C_t, we get

$$\varepsilon_A^1 f_A + \varepsilon_B^1 f_B^1 + \varepsilon_C^1 f_C = A^1/C_t \tag{24}$$

$$\varepsilon_A^2 f_A + \varepsilon_B^2 f_B + \varepsilon_C^2 f_C = A^2/C_t \tag{25}$$

$$\varepsilon_A^3 f_A + \varepsilon_B^3 f_B + \varepsilon_C^3 f_C = A^3/C_t \tag{26}$$

where

$$f_A + f_B + f_C = 1 \tag{27}$$

If we treat C_t as a known quantity, the system is over-determined, and we can eliminate one equation, say equation (26). Then the remaining equations can be expressed in matrix form as

$$\begin{vmatrix} \varepsilon_A^1 & \varepsilon_B^1 & \varepsilon_C^1 \\ \varepsilon_A^2 & \varepsilon_B^2 & \varepsilon_C^2 \\ 1 & 1 & 1 \end{vmatrix} \begin{vmatrix} f_A \\ f_B \\ f_C \end{vmatrix} = \frac{1}{C_t} \begin{vmatrix} A^1 \\ A^2 \\ C_t \end{vmatrix} \tag{28}$$

Thus measurements at only two wavelengths plus the value of C_t are required for the analysis (C_t is determined as shown on page 9).

With the total concentration of the mixture known, two wavelengths are chosen at which the relative absorptivities

of the three components vary. A^1/C_t and A^2/C_t as well as the six absorptivities of the components are determined at these two wavelengths. A^1/C_t can be thought of as an apparent absorptivity ε_S^1 and A^2/C_t as ε_S^2. All of these quantities are now comparable, because they are on the same (molar) basis.

The corresponding pairs of 'calibrating' ε^1 and ε^2 values for each component, are entered at the vertices of the triangle. (ε^2 values will be underlined so that they will be distinguished from ε^1 values.) ε_S^1 will have a value between (say) ε_A^1 and ε_C^1. By simple linear interpolation, a point between ε_A^1 and ε_C^1 corresponding to this value is found. Similarly, if ε_S^1 falls between ε_B^1 and ε_C^1, the point corresponding to it is found. The two points are then connected by a straight line (iso-absorptivity line). ε_S^2 is treated similarly and the points are connected.

The meaning of these tie lines is that all solutions of compositions falling on one of these lines have ε_S values identical with the ones plotted. Therefore the point of intersection of the two lines is the solution that can have both ε_S^1 and ε_S^2 values, and this is the sample composition. With this composition known, a reference solution with that composition can be prepared and its spectrum compared (matched) with that of the given sample.

For a four-component mixture, a similar procedure is followed. It is however important to note that for a

three-component mixture, analysis was possible by using two wavelengths and a two-dimensional plot. In an analogous way, we can analyze a four-component mixture by solving the set of equations

$$f_A + f_B + f_C + f_D = 1 \tag{29}$$

$$\varepsilon_A^1 f_A + \varepsilon_B^1 f_B + \varepsilon_C^1 f_C + \varepsilon_D^1 f_D = A^1/C_t \tag{30}$$

$$\varepsilon_A^2 f_A + \varepsilon_B^2 f_B + \varepsilon_C^2 f_C + \varepsilon_D^2 f_D = A^2/C_t \tag{31}$$

and

$$\varepsilon_A^3 f_A + \varepsilon_B^3 f_B + \varepsilon_C^3 f_C + \varepsilon_D^3 f_D = A^3/C_t \tag{32}$$

which can be put in matrix notation as

$$\begin{vmatrix} 1 & 1 & 1 & 1 \\ \varepsilon_A^1 & \varepsilon_B^1 & \varepsilon_C^1 & \varepsilon_D^1 \\ \varepsilon_A^2 & \varepsilon_B^2 & \varepsilon_C^2 & \varepsilon_D^2 \\ \varepsilon_A^3 & \varepsilon_B^3 & \varepsilon_C^3 & \varepsilon_D^3 \end{vmatrix} \begin{vmatrix} f_A \\ f_B \\ f_C \\ f_D \end{vmatrix} = \frac{1}{C_t} \begin{vmatrix} C_t \\ A^1 \\ A^2 \\ A^3 \end{vmatrix} \tag{33}$$

This can be solved by using a three-dimensional plot and data at three wavelengths, if the total concentration is known. The three-dimensional figure required for this plot will be a regular tetrahedron. In this figure, each of the

four apices represents a pure component; the six edges represent the six possible binary mixtures; the four sides are the four ternary mixtures, and any point inside the tetrahedron represents a four-component mixture. The side opposite each apex represents zero fraction of that substance and its fractional composition increases from this opposite side to the apex.

In a manner similar to the three-component analysis, the twelve absorptivities of the four components are obtained at three wavelengths (one at each wavelength). The absorptions of the sample at the chosen wavelengths are also found. The chosen wavelengths are those at which the ratios of the absorptivities vary. The absorptivities of each of the components are entered on each of the apices of the tetrahedron. By linear interpolation, the 'apparent absorptivity' A^1/C_t is marked by a pin between (say) ε_A^1 and ε_B^1. This is done similarly for the other edges when possible. All the pins are then connected by a thread, which defines an "iso-absorptive" plane. The same is done for A^2/C_t and A^3/C_t. Threads of different colors are used to differentiate values corresponding to different wavelengths. Corresponding points of intersection of a pair of threads are connected by a wire. The same thing is done for the other pairs of intersecting lines. The point of intersection of the wires gives the sample composition. That is, any two planes intersect to define a line, and the intersection of

this line with a third plane defines a point, which corresponds to the sample.

Mid-Range and Local Solutions

In the mid-range solutions, reference measurements are made on three or four systems defining a triangle or tetrahedron whose area or volume is about one-quarter to one-half of that of the entire diagram for three- and four-component mixtures respectively. One or two of the reference systems may be pure components, or all may be mixtures; the reference compositions are chosen such that the sample composition is included within the reference triangle or tetrahedron. This solution sacrifices some accuracy in location of the sample iso-absorbance contour because the differences along the sides are smaller than in the full-range solution. However, there is gain in freedom from distortion due to non-ideality, because the calibrating reference systems are closer to the sample system.

In the local solution, the reference system on which the measurements are made should define a triangle or tetrahedron approximately one-tenth to one-twentieth of the area or volume of the full-range solution, and including the sample composition. A local solution minimizes errors from non-ideal behavior, but admits greater uncertainty in locating the iso-absorbance contour. Mid-range and local solutions were developed for the kinetic analysis method (4),

but have not been used in the present work because absorption spectra usually exhibit additive behavior.

C. Determination of Acid Dissociation Constants

UV-visible spectrophotometry is one of the techniques that has been used for the determination of dissociation constants of acids (30). Cookson (31) has given a good review of this method. For an acid HA, the equilibrium (34) in aqueous solution can be written

$$HA \rightleftarrows H^+ + A^- \tag{34}$$

The thermodynamic acid dissociation constant is defined as

$$K_a = \frac{a_{H^+}a_{A^-}}{a_{HA}} = \frac{c_{H^+}c_{A^-}y_{\pm}^2}{c_{HA}y_{HA}} \tag{35}$$

where a_i and c_i are activity and concentration of component i, $y_{\pm}$ is the mean ionic activity coefficient of H^+ and A^-, and y_{HA} is the activity coefficient of HA. The apparent dissociation constant is defined as

$$K_a' = \frac{a_{H^+}c_{A^-}}{c_{HA}} \tag{36}$$

at low ionic strengths where equality of the activity and concentration of the neutral species [HA] occurs. Taking logarithms of equations (35) and (36) give

$$pK_a = pH - \log \frac{a_{A^-}}{a_{HA}} \tag{37}$$

and

$$pK_a' = pH - \log \frac{c_{A^-}}{c_{HA}} \tag{38}$$

respectively.

Thus by knowing c_{A^-} and c_{HA} at any pH, pK_a' can be determined. At any given pH, where A^- and HA are present, we can write

$$A_t = \varepsilon_{HA} c_{HA} + \varepsilon_{A^-} c_{A^-} \tag{39}$$

hence

$$\frac{A_t}{\varepsilon_{HA}} = c_{HA} + \frac{\varepsilon_{A^-}}{\varepsilon_{HA}} c_{A^-} \tag{40}$$

Thus at any pH, if A^- and HA have different spectra, by using equation (40) to determine c_{A^-} and c_{HA}, pK_a' can be obtained.

The apparent dissociation constant depends on the ionic strength of the solution. To obtain the correct dissociation constant, correction has to be made for the effect of ionic strength on K_a'. At low ionic strengths and using Debye-Hückel theory, for neutral acids in aqueous solution,

$$pK_a = pK_a' + \frac{0.509\sqrt{I_S}}{1 + \sqrt{I_S}} \qquad (41)$$

where I_S is the ionic strength of the solution (14).

III. EXPERIMENTAL

A. Materials

All inorganic materials were used without further purification. Sodium hydroxide, potassium chloride, and sodium borate (Mallinckrodt) were of analytical reagent grade. Potassium biphthalate (Baker Chemical Company) was Baker Analyzed Reagent, Primary Standard. Hydrochloric acid (Allied Chemical Corporation) met ACS specifications.

Salicylic acid (Fred Portz) was recrystallized from water; melting (m.p.) 159-160°C, literature (lit.) value 159.5-160.5°C (32). Acetylsalicylic acid (Merck) was recrystallized from acetone; m.p. 135-136°C, lit. 135°C (33). Tetracaine hydrochloride (Pfaltz and Bauer) was recrystallized from water; m.p. 147-148°C, lit. 147-149°C (34). Benzocaine (Aldrich) was recrystallized from aqueous ethanolic solution; m.p. 89-90°C, lit. 89-90°C (35). Sulfathiazole and sulfanilamide (Merck) were recrystallized from alcohol; m.p. 202-203°C, 164-165°C, lit. 202-202.5°C and 163-164°C (36,37) respectively. p-Hydroxybenzoic acid (Eastman) was recrystallized from water; m.p. 213-214°C, lit. 214-215°C (38). 2,4-Dihydroxypyridine and 2,3-pyridine dicarboxylic acid (Aldrich) were recrystallized from aqueous ethanolic solutions; m.p. 263-264°C, 190°C, lit. 260-265°C, 190°C (39, 40) respectively.

The following compounds were used without further

purification. Benzoic acid (Mallinckrodt) was analytical reagent grade; sodium acetate (Baker) met ACS specifications; m-hydroxybenzoic acid (Pfaltz and Bauer); 2,6-pyridine dicarboxylic acid; 2,3-dihydroxypyridine; 2,4-dichlorophenol; 2,5-dichlorophenol; 2,6-dichlorophenol; all obtained from Aldrich; and 95% ethanol (International Minerals and Chemical Corporation) was USP grade.

Standard buffers were prepared according to Bates (41-42) and Weast (43).

B. Apparatus

Spectrophotometric measurements were made with the Varian Cary 14 spectrophotometer fitted with thermostated cell compartment that maintained temperature constant at 25 ± 0.1°C. The pH measurements were made with an Orion Model 801 pH meter. Water bath temperature was maintained constant within ±0.1°C with a Sargent Thermonitor Electronic relay. Thermometers were calibrated with a certified thermometer. Melting points were determined with a Thomas Hoover capillary melting point apparatus.

C. Procedures

Synthetic mixtures were prepared from stock solutions of known concentrations of the individual components. For benzocaine and tetracaine hydrochloride, and for sulfathiazole and sulfanilamide mixtures, 95% ethanol was used as solvent.

For benzoic acid, salicylic acid, and p-hydroxybenzoic acid; and for salicylic acid, benzoic acid, p-hydroxybenzoic acid, and m-hydroxybenzoic acid mixtures, approximately 0.02 M NaOH was used as solvent. For 2,3-dihydroxypyridine, 2,3-pyridine dicarboxylic acid, and 2,6-pyridine dicarboxylic acid; for 2,4-dichlorophenol, 2,5-dichlorophenol, and 2,6-dichlorophenol; for 2,4-dihydroxypyridine, 2,3-dihydroxy-pyridine, 2,3-pyridine dicarboxylic acid, and 2,6-pyridine dicarboxylic acid mixtures, water:95% ethanol (50:50) was used as solvent; while for salicylic acid and acetylsalicylic acid mixtures, chloroform was the solvent.

In the determination of dissociation constants of acids, buffer solutions of different pH were prepared by using hydrochloric acid, sodium acetate, and sodium hydroxide. By pipetting equal volumes of stock solutions of the acids into each of the buffer solutions and diluting to mark, total solute concentration was maintained constant in all the solutions. For benzoic acid, this was 7.5×10^{-5} M, whereas for trans-cinnamic acid, it was 4.5×10^{-5} M. Reference solutions as blanks were prepared for each solution by omitting the acid. The pH meter was calibrated against phthalate and borate buffers. All the solutions were thermostated at 25°C before measurements were made.

IV. RESULTS

A. Analysis of Two- and Three-Component Mixtures Using Entire Spectral Range

Sulfathiazole and Sulfanilamide Analysis

The spectra of the pure components are shown in Fig. 1. Figs. 2 and 3 are examples of the different plots for calculating the individual absolute concentrations for the same sample in two-component mixtures.

Results obtained from the plots of

$$\frac{A_t}{\varepsilon_{SM}} = C_{SM} + \frac{\varepsilon_{ST}}{\varepsilon_{SM}} C_{ST} \qquad (42)$$

as in Fig. 2 are shown in Table I. Sulfathiazole [ST] concentrations were obtained from the slopes whereas those of sulfanilamide [SM] were obtained from the intercepts. From statistical analysis[a], the mean percentage recovery for sulfathiazole is (99.3 $\pm$ 1.4)% for concentrations greater than 5.2×10^{-6} M, N = 9; whereas for sulfanilamide concentrations greater than 6.3×10^{-6} M, mean percentage recovery is (101.3 $\pm$ 2.4)% and N = 7.

Results obtained from the plots of

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[a]All the statistical analysis reported are at 95% confidence level. The stated uncertainty represents the confidence limits based on Student's t distribution.

Figure 1. Spectra of sulfathiazole [ST] and sulfanilamide [SM] in 95% ethanol.

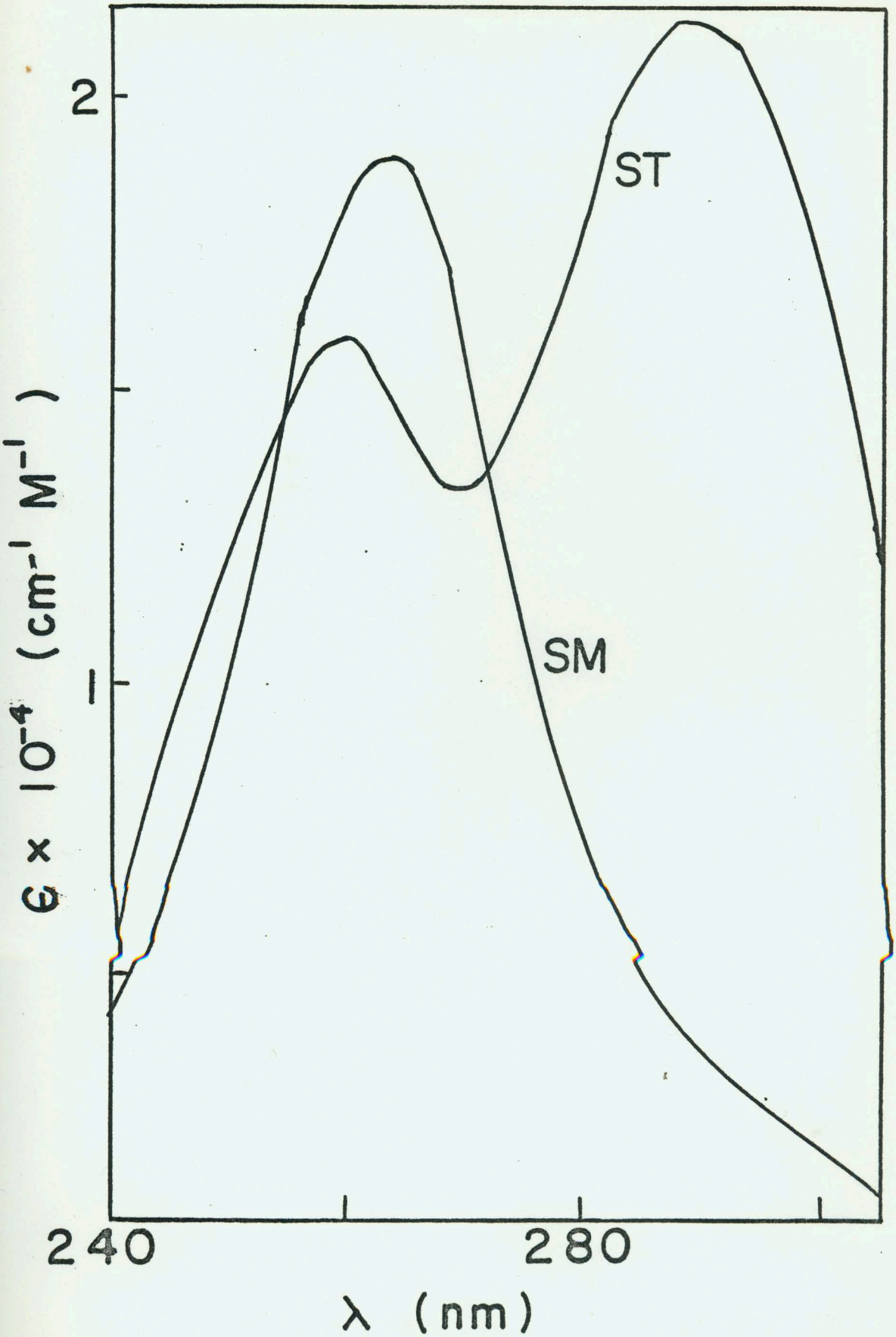

2
1
ε x 10⁻⁴ (cm⁻¹ M⁻¹)
ST
SM
240
280
λ (nm)

Figure 2. Plot of equation (42) used in obtaining the individual concentrations of sulfathiazole and sulfanilamide as an example of determining the absolute concentrations of the individual components in a binary mixture.

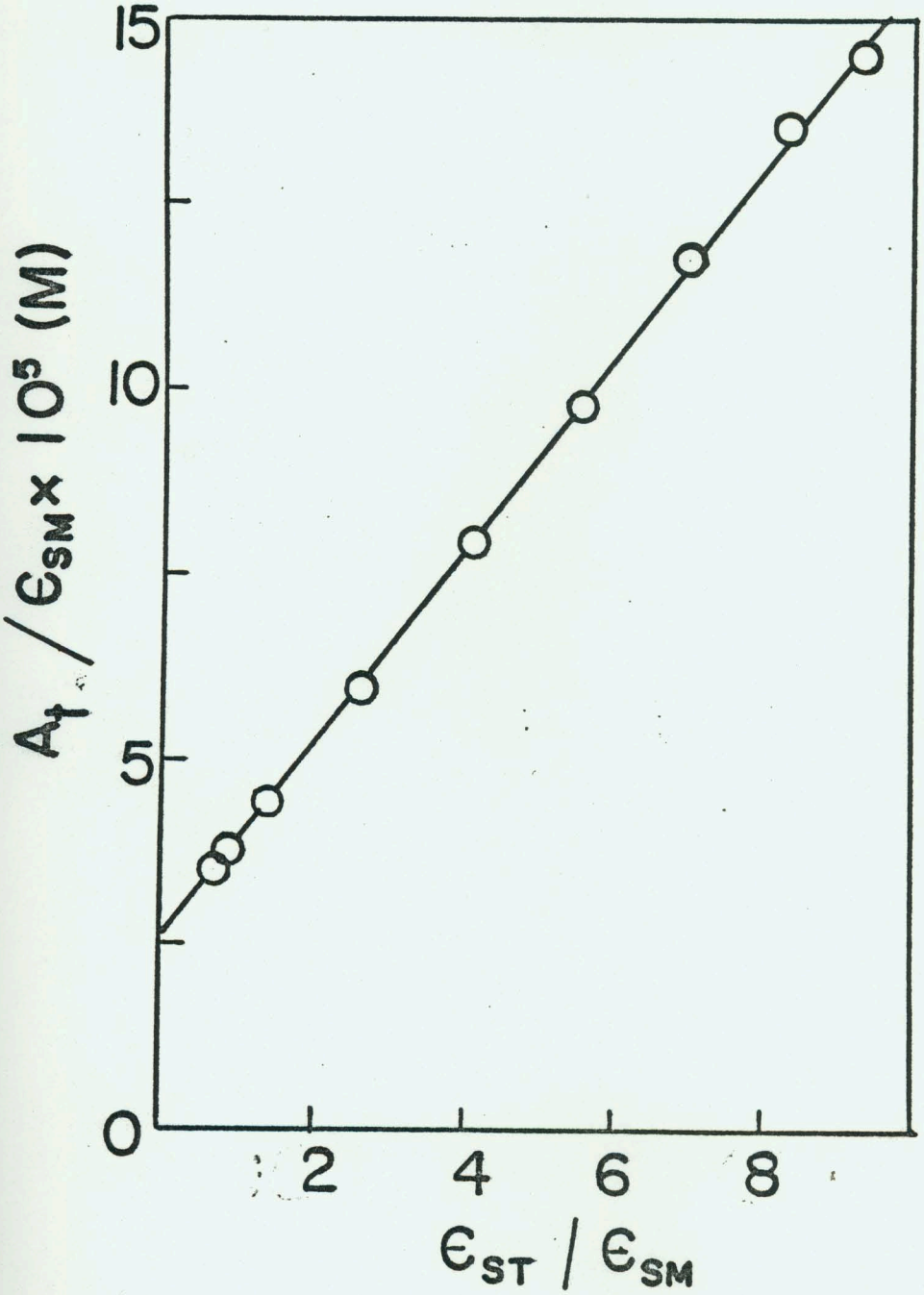

15
10
5
0
2
4
6
8
$A_t / \epsilon_{SM} \times 10^5$ (M)
$\epsilon_{ST} / \epsilon_{SM}$

Figure 3. Plot of equation (43) used in obtaining the individual concentrations of sulfathiazole and sulfanilamide as an alternative example of determining the absolute concentrations of a binary mixture.

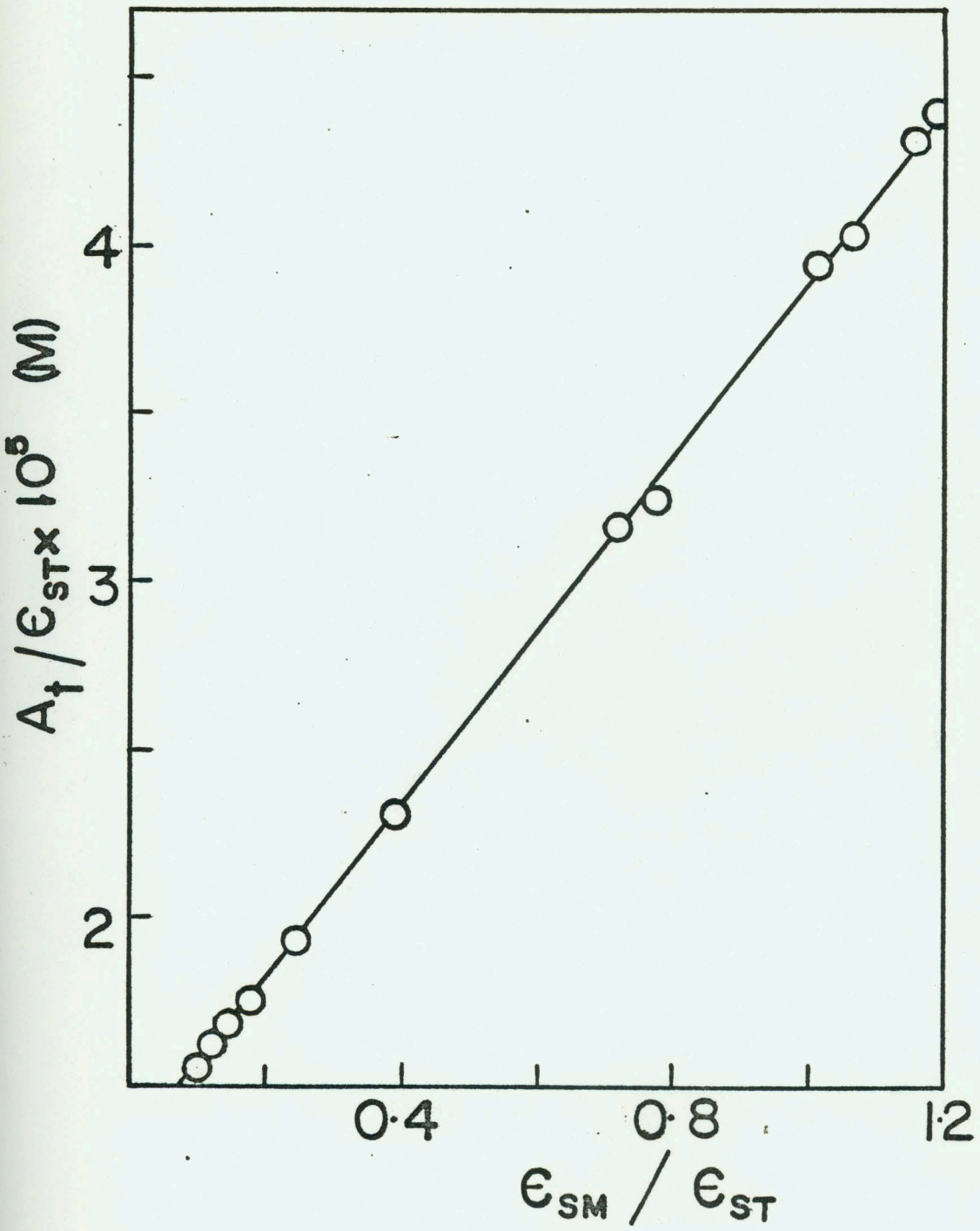

$A_t / \epsilon_{ST} \times 10^5$ (M)
2
3
4
0·4
0·8
1·2
$\epsilon_{SM} / \epsilon_{ST}$

Table I. Sulfathiazole and Sulfanilamide Analysis[a]

Sample	Sulfathiazole concn x 10^5 M		% Recovery	Sulfanilamide concn x 10^5 M		% Recovery
	Taken	Found		Taken	Found	
1	2.16	2.18	100.8	2.09	2.02	96.5
2	2.16	2.15	99.5	1.57	1.65	104.9
3	2.16	2.11	97.6	1.05	1.09	104.1
4	2.16	2.13	98.2	0.63	0.63	99.6
5	2.16	2.14	99.0	0.42	0.56	133.1
6	2.16	2.20	101.7	0.21	0.20	95.6
7	2.60	2.58	99.4	0.42	0.52	123.9
8	1.30	1.28	98.4	2.62	2.62	100.3
9	0.87	0.84	97.2	2.62	2.71	103.6
10	0.52	0.48	93.2	2.62	2.68	102.3
11	0.35	0.34	98.6	2.62	2.61	99.4

[a]Results obtained from plots of equation (42).

Table II. Sulfathiazole and Sulfanilamide Analysis[a]

Sample	Sulfanilamide concn x 10^5 M		% Recovery	Sulfathiazole concn x 10^5 M		% Recovery
	Taken	Found		Taken	Found	
1	2.09	2.04	97.5	2.16	2.17	100.3
2	1.57	1.59	101.1	2.16	2.14	99.0
3	1.05	1.05	100.1	2.16	2.12	97.9
4	0.63	0.65	103.1	2.16	2.13	98.3
5	0.42	0.51	121.0	2.16	2.14	99.1
6	0.21	0.23	110.9	2.16	2.18	100.7
7	0.42	0.51	122.7	2.60	2.57	99.0
8	2.62	2.60	99.4	1.30	1.30	100.1
9	2.62	2.64	101.1	0.87	0.87	100.2
10	2.62	2.63	100.5	0.52	0.50	95.6
11	2.62	2.72	104.0	0.35	0.32	92.4

[a]Results obtained from plots of equation (43).

$$\frac{A_t}{\varepsilon_{ST}} = C_{ST} + \frac{\varepsilon_{SM}}{\varepsilon_{ST}} C_{SM} \qquad (43)$$

as in Fig. 3 are shown in Table II. Sulfathiazole concentrations were obtained from the intercepts whereas those of sulfanilamide were obtained from the slopes. For sulfanilamide concentrations greater than 6.3×10^{-6} M, statistical analysis gives mean percentage recovery as $(100.9 \pm 1.7)\%$, N = 7; whereas for sulfathiazole, mean percentage recovery is $(99.3 \pm 0.8)\%$ and N = 9 for concentrations greater than 5.2×10^{-6} M. Data used in plots were obtained from 240 to 305 nm.

Benzocaine and Tetracaine Analysis

The spectra of the pure components are shown in Fig. 4. Results obtained from the plots of

$$\frac{A_t}{\varepsilon_T} = C_T + \frac{\varepsilon_B}{\varepsilon_T} C_B \qquad (44)$$

are shown in Table III. Thus concentrations of tetracaine [T] were obtained from the intercepts whereas those of benzocaine [B] were obtained from the slopes. From statistical analysis of the results, mean percentage recovery for tetracaine is $(99.0 \pm 0.9)\%$, N = 7 for concentrations greater than 10^{-5} M; whereas for benzocaine, mean percentage recovery is $(102.2 \pm 3.2)\%$, N = 8 for concentrations greater than 1.6×10^{-5} M.

Figure 4. Spectra of tetracaine hydrochloride [T] and benzocaine [B] in water:95% ethanol (50:50).

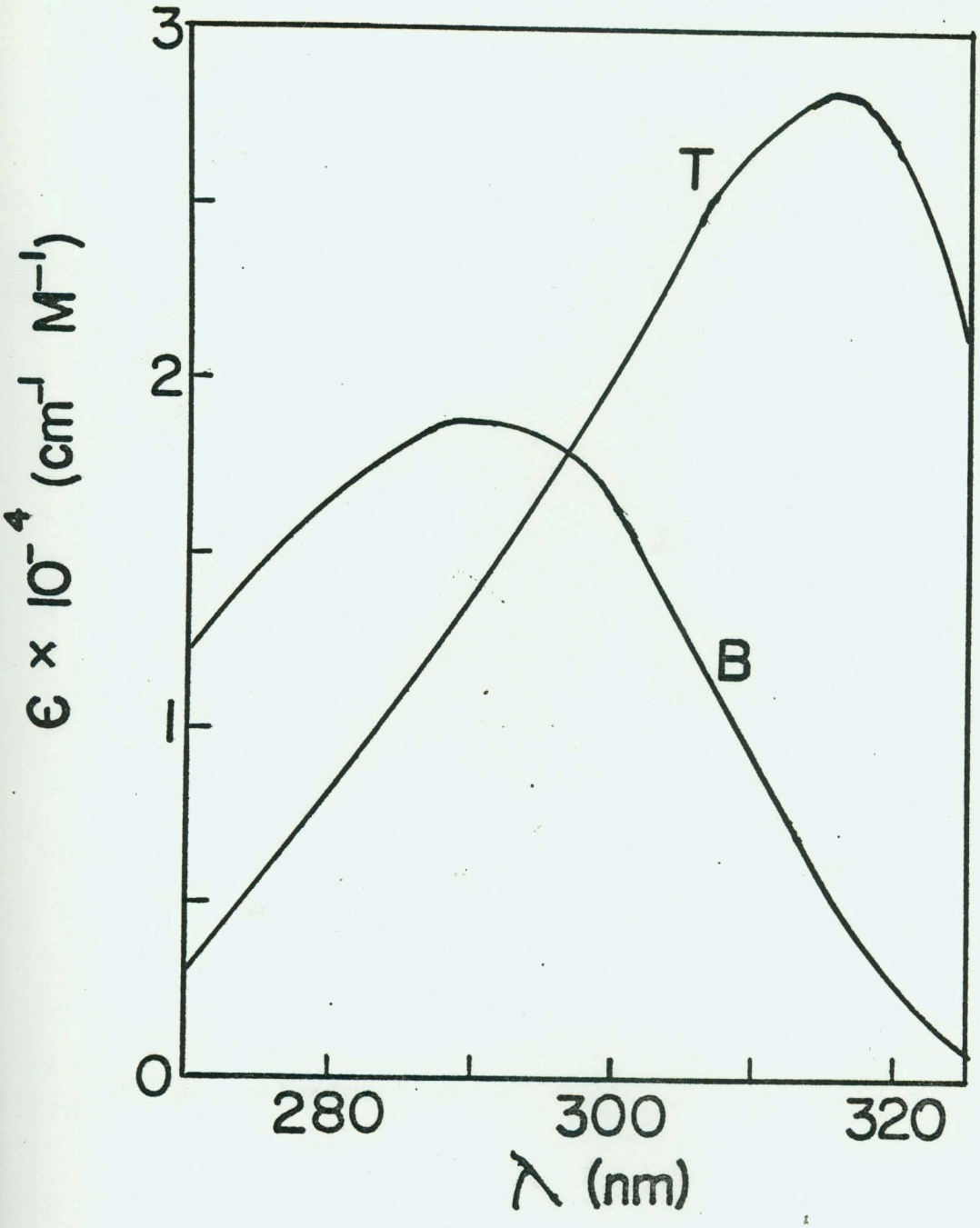

3
2
1
0
T
B
280
300
320
λ (nm)
ε × 10⁻⁴ (cm⁻¹ M⁻¹)

Table III. Benzocaine and Tetracaine Analysis[a]

Sample	Tetracaine concn x 10^5 M		% Recovery	Benzocaine concn x 10^5 M		% Recovery
	Taken	Found		Taken	Found	
1	1.41	1.38	97.8	1.50	1.57	104.6
2	1.41	1.39	98.9	1.12	1.26	112.2
3	1.41	1.42	101.0	0.75	0.80	106.9
4	1.41	1.39	98.9	0.45	0.53	118.1
5	1.41	1.39	98.9	0.30	0.32	107.3
6	1.41	1.37	97.7	0.15	0.18	117.9
7	0.84	0.80	94.8	1.87	1.80	96.2
8	0.56	0.53	93.6	1.87	1.79	95.9
9	0.34	0.33	98.7	1.87	1.87	100.2
10	0.23	0.24	104.3	1.87	1.91	102.1
11	0.11	0.14	127.6	1.87	1.96	104.6
12	1.69	1.69	100.1	0.15	0.26	174.8
13	0.23	0.28	125.9	2.62	2.67	101.8

[a]Results obtained from plot of equation (44).

Results obtained from the plots of

$$\frac{A_t}{\varepsilon_B} = C_B + \frac{\varepsilon_T}{\varepsilon_B} C_T \qquad (45)$$

are shown in Table IV. Concentrations of tetracaine were obtained from the slopes whereas those of benzocaine were obtained from the intercepts. From statistical analysis, for concentrations of tetracaine greater than 10^{-5} M, mean percentage recovery is (99.3 $\pm$ 0.9)%, N = 7; whereas for concentrations of benzocaine greater than 1.6×10^{-5} M, mean percentage recovery is (100.8 $\pm$ 3.3)% and N = 8. Data used in plots for calculating the concentrations were in the range of 265 to 325 nm.

Salicylic Acid and Aspirin Analysis

The spectra of the pure components are shown in Fig. 5. Results obtained from the plots of

$$\frac{A_t}{\varepsilon_{ASA}} = C_{ASA} + \frac{\varepsilon_{SA}}{\varepsilon_{ASA}} C_{SA} \qquad (46)$$

are shown in Table V. Concentrations of salicylic acid [SA] were obtained from the slopes whereas those of aspirin [ASA] were obtained from the intercepts. From statistical analysis, for concentrations of salicylic acid greater than 2.5×10^{-5} M, mean percentage recovery is (99.3 $\pm$ 3.1)%, N = 5; whereas for aspirin (concentrations fixed at 3.7×10^{-4} M and 3.8×10^{-4} M), mean percentage recovery is (98.6 $\pm$ 1.2)%

Table IV. Benzocaine and Tetracaine Analysis[a]

Sample	Tetracaine concn x 10^5 M		% Recovery	Benzocaine concn x 10^5 M		% Recovery
	Taken	Found		Taken	Found	
1	1.41	1.39	98.5	1.50	1.58	105.2
2	1.41	1.41	99.9	1.12	1.23	109.2
3	1.41	1.42	101.1	0.75	0.83	110.8
4	1.41	1.40	99.7	0.45	0.55	122.2
5	1.41	1.37	97.3	0.30	0.35	117.5
6	1.41	1.39	98.8	0.15	0.16	107.7
7	0.84	0.80	94.2	1.87	1.80	96.0
8	0.56	0.51	90.4	1.87	1.82	97.4
9	0.34	0.33	98.4	1.87	1.83	97.7
10	0.23	0.26	114.1	1.87	1.84	98.5
11	0.11	0.16	145.9	1.87	1.90	101.6
12	1.69	1.69	100.0	0.15	0.31	208.8
13	0.23	0.26	117.1	2.62	2.62	100.7

[a]Results obtained from plots of equation (45).

Figure 5. Spectra of aspirin [ASA] and salicylic acid [SA] in chloroform.

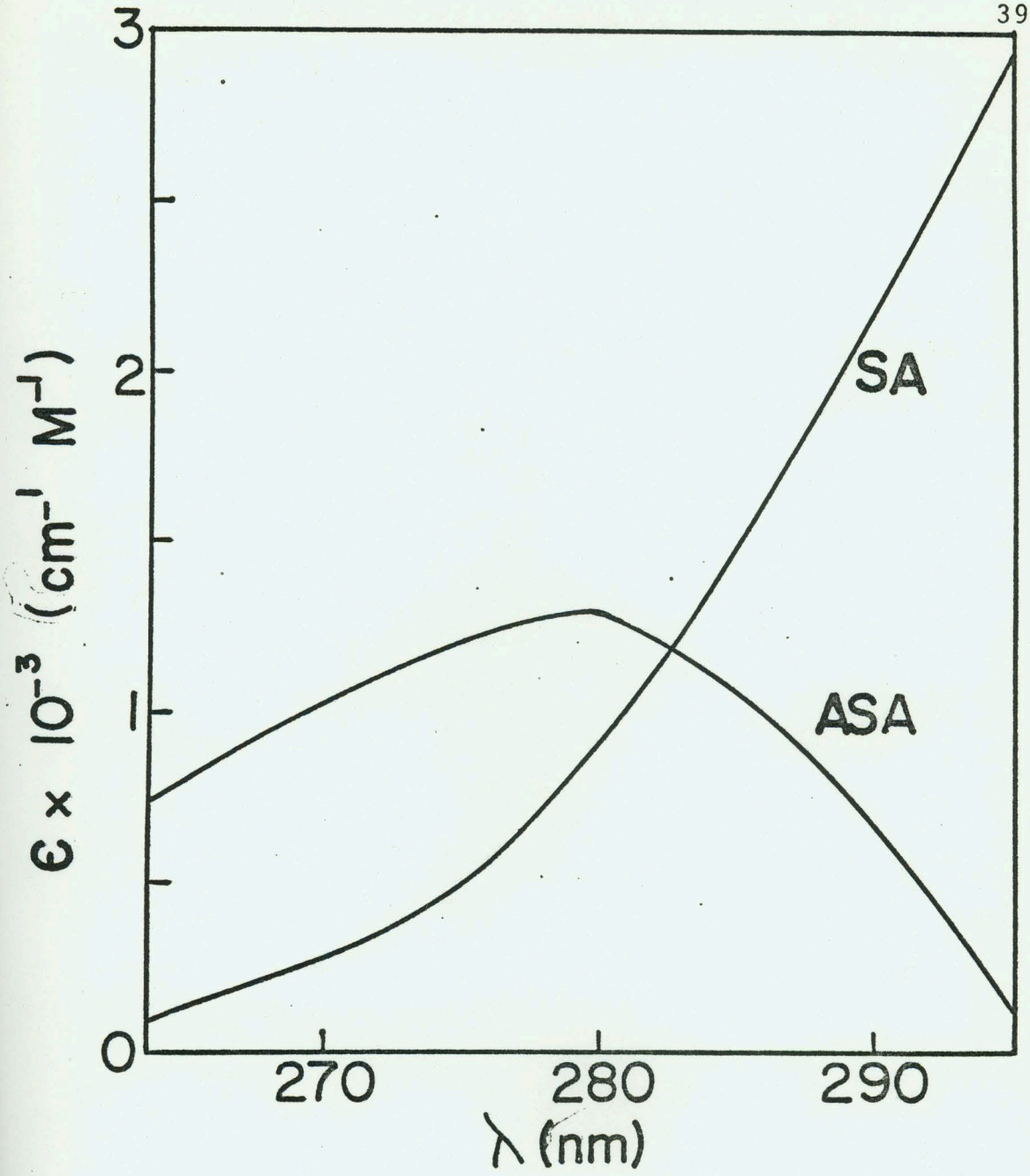

3
2
1
0
ε x 10⁻³ (cm⁻¹ M⁻¹)
SA
ASA
270
280
290
λ (nm)

Table V. Salicylic Acid and Aspirin Analysis[a]

Sample	Salicylic Acid concn x 10^5 M		% Recovery	Aspirin concn x 10^4 M		% Recovery
	Taken	Found		Taken	Found	
1	4.13	4.00	96.8	3.74	3.72	99.4
2	2.48	2.43	97.9	3.74	3.65	97.4
3	1.65	1.54	93.1	3.74	3.72	98.4
4	1.24	1.10	89.0	3.74	3.68	98.2
5	0.83	0.74	89.0	3.74	3.72	99.4
6	6.41	6.44	100.4	3.83	3.83	100.0
7	4.28	4.21	98.5	3.83	3.65	95.5
8	2.57	2.65	103.1	3.83	3.84	100.3
9	1.71	1.34	78.6	3.83	3.66	95.8
10	1.28	1.08	84.4	3.83	3.75	98.1
11	0.43	0.45	105.3	3.83	3.86	100.9

[a]Results obtained from plot of equation (46).

and N = 11.

Results obtained from the plots of

$$\frac{A_t}{\varepsilon_{SA}} = C_{SA} + \frac{\varepsilon_{ASA}}{\varepsilon_{SA}} C_{ASA} \qquad (47)$$

are shown in Table VI. Concentrations of salicylic acid were obtained from the intercepts whereas those of aspirin were obtained from the slopes. From statistical analysis, for concentrations of salicylic acid greater than 1.7×10^{-5} M, mean percentage recovery is (100.7 $\pm$ 1.3)%, N = 7; whereas for concentrations of aspirin (fixed at 3.7×10^{-4} M and 3.8×10^{-4} M), mean percentage recovery is (97.8 $\pm$ 1.2)% and N = 13. Data for plots used in the determination of the concentrations were obtained from 260 to 295 nm.

Summary of Two-Component Mixtures

Table VII summarizes the results of analyses on two-component mixtures analyzed by using data over a wide spectral range along with those obtained by conventional simultaneous equations method.

2,6-Pyridine dicarboxylic Acid, 2,3-Pyridine dicarboxylic Acid, and 2,3-Dihydroxypyridine Analysis

The spectra of the pure components are shown in Fig. 6. An example of the plot for the determination of total concentration in ternary systems is shown in Fig. 7. Readings at the iso-absorptive points for 2,6-pyridine dicarboxylic acid

Table VI. Salicylic Acid and Aspirin Analysis[a]

	Salicylic Acid concn x 10^5 M			Aspirin concn x 10^4 M		
Sample	Taken	Found	% Recovery	Taken	Found	% Recovery
1	4.13	4.18	101.1	3.74	3.69	98.7
2	2.48	2.55	102.8	3.74	3.62	96.6
3	1.65	1.66	100.3	3.74	3.71	99.2
4	1.24	1.44	116.5	3.74	3.56	95.0
5	0.83	0.84	101.0	3.74	3.68	98.2
6	0.11	0.35	84.9	3.74	3.59	95.8
7	6.41	6.50	101.3	3.83	3.80	99.3
8	4.28	4.26	99.6	3.83	3.71	97.1
9	2.57	2.53	98.6	3.83	3.86	100.8
10	1.71	1.73	101.3	3.83	3.67	95.8
11	1.28	1.32	102.9	3.83	3.72	97.2
12	0.86	0.67	77.9	3.83	3.68	96.1
13	0.43	0.93	218.3	3.83	3.87	101.1

[a]Results obtained from plots of equation (47).

Table VII. Summary of Two-Component Mixtures

Sample Mixture	% Recovery from Slope	N	Min. Conc. Determined	% Recovery from Intercept	N	Min. Conc. Determined	% Recovery from Simultaneous Equation[a]
Benzocaine	100.8 ± 3.3	7	1.6×10^{-5} M	99.6 ± 2.9	7	1.6×10^{-5} M	101.1 ± 3.4
Tetracaine	99.3 ± 0.9	7	1×10^{-5} M	99.0 ± 0.9	7	1×10^{-5} M	99.7 ± 1.4
Sulfathiazole	99.3 ± 1.4	9	8.7×10^{-6} M	99.3 ± 0.8	9	8.7×10^{-6} M	98.9 ± 1.1
Sulfanilamide	100.9 ± 1.7	7	6.3×10^{-6} M	101.3 ± 2.4	7	6.3×10^{-6} M	99.5 ± 1.1
Salicylic acid	99.3 ± 3.1	5	2.5×10^{-5} M	100.7 ± 1.3	7	1.7×10^{-5} M	98.0 ± 6.1
Aspirin	97.8 ± 1.2	13	Fixed at 3.7 and 3.8 x 10^{-4} M	98.6 ± 1.2	13	Fixed at 3.7 and 3.8 x 10^{-4} M	97.0 ± 1.7

[a]Data treatment by conventional solution of simultaneous equations based on measurements at two wavelengths.

Figure 6. Spectra of 2,6-pyridine dicarboxylic acid [DPA], 2,3-pyridine dicarboxylic acid [PDA] and 2,3-dihydroxy-pyridine [3DHP] in 95% ethanol.

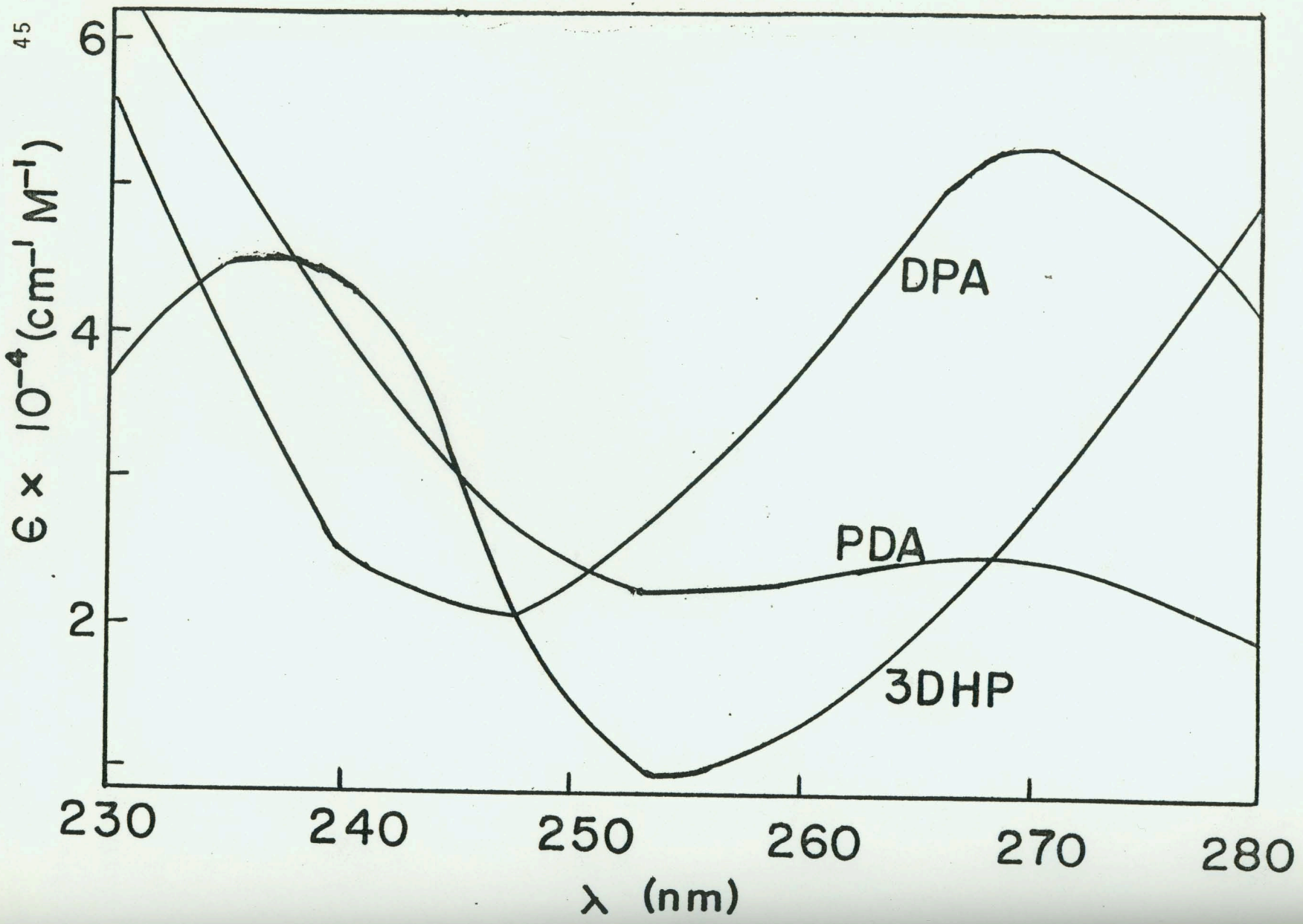
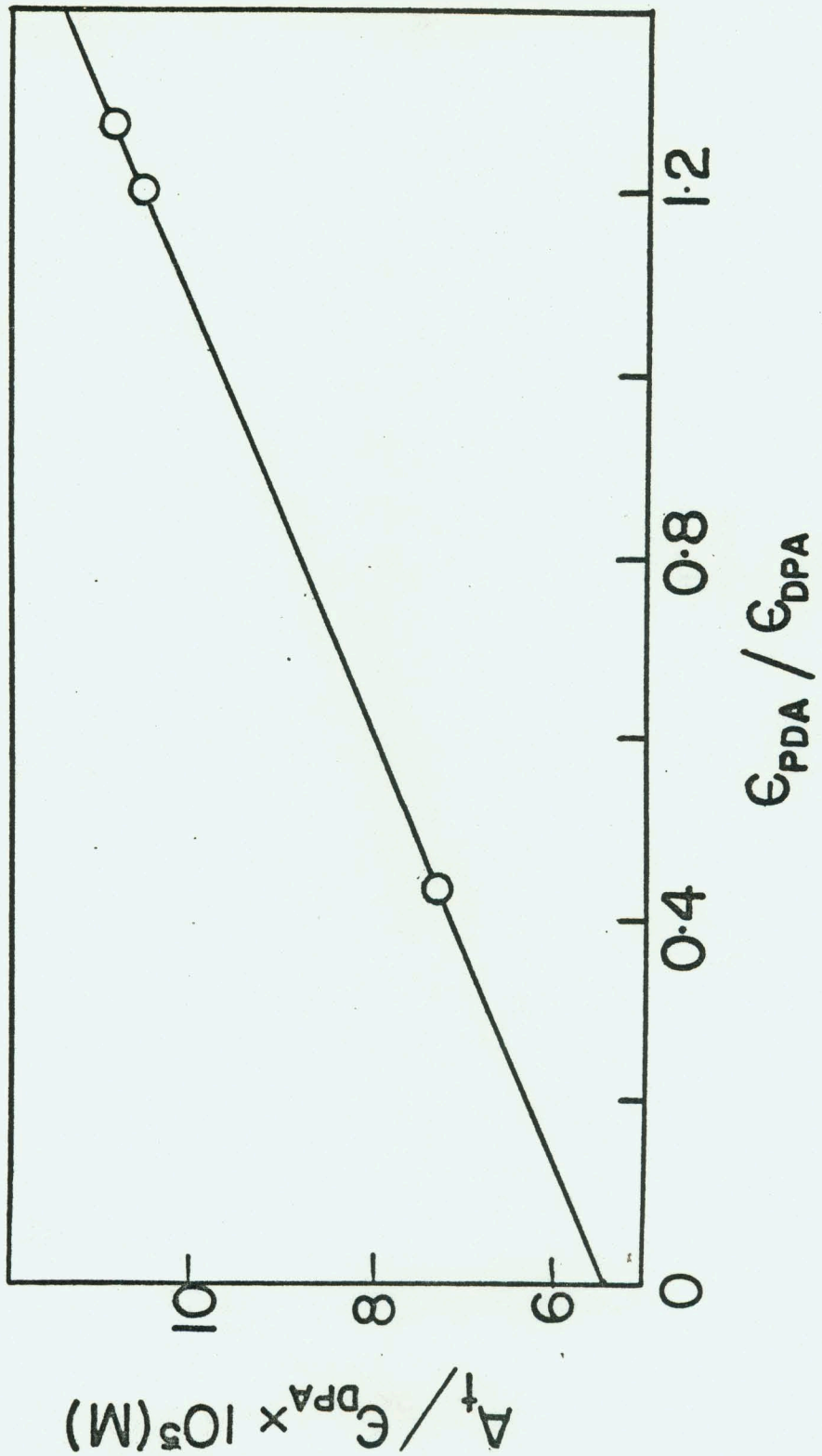

6
4
2
ε x 10⁻⁴ (cm⁻¹ M⁻¹)
DPA
PDA
3DHP
230
240
250
260
270
280
λ (nm)

Figure 7. Plot of equation (48) as an example of determining the total concentration of a sample in ternary mixtures using readings at the iso-absorptive points of a pair of the components. Data plotted are for sample 7, Table VIII.


$A_t / \epsilon_{DPA} \times 10^5 (M)$
10
8
6
0
0·4
0·8
1·2
$\epsilon_{PDA} / \epsilon_{DPA}$

[DPA] and 2,3-dihydroxypyridine [3DHP] at 234, 247.8 and 278.3 nm were used for the plots using equation (48)

$$\frac{A_t}{\varepsilon_{DPA}} = (C_{DPA} + C_{3DHP}) + \frac{\varepsilon_{PDA}}{\varepsilon_{DPA}} C_{PDA} \qquad (48)$$

Results from such plots are shown in Table VIII, and from statistical analysis, mean percentage recovery of the total concentration is (100.1 $\pm$ 0.8)%; N = 10.

An example of the plot for the determination of the fractional concentrations in ternary systems using the entire spectral range using equation (49), is shown in Fig. 8.

$$\frac{(A_t/C_t) - \varepsilon_{3DHP}}{\varepsilon_{PDA} - \varepsilon_{3DHP}} = f_{PDA} + \frac{\varepsilon_{DPA} - \varepsilon_{3DHP}}{\varepsilon_{PDA} - \varepsilon_{3DHP}} f_{DPA} \qquad (49)$$

For all concentrations greater than 1.1×10^{-5} M, overall mean percentage recovery is (100.3 $\pm$ 1.5)%, N = 27; for 2,3-pyridine dicarboxylic acid (98.2 $\pm$ 2.8)%, N = 9; for 2,6-pyridine dicarboxylic acid (102.0 $\pm$ 3.6)%, N = 9; and for 2,3-dihydroxypyridine (100.9 $\pm$ 2.3)%, N = 9. The range of wavelength used for the plots is 260 to 285 nm.

2,4-Dichlorophenol, 2,5-Dichlorophenol, and 2,6-Dichlorophenol Analysis

The spectra of the pure components are shown in Fig. 9. Readings at the iso-absorptive points for 2,4-dichlorophenol [24], and 2,5-dichlorophenol [25] at 286.3, 289.0 and

Table VIII. Total Concentrations of 2,3-Dihydroxypyridine, 2,3-Pyridine dicarboxylic Acid and 2,6-Pyridine dicarboxylic Acid Mixtures[a]

	Total Concentration x 10^5 M		
Sample	Taken	Found	% Recovery
1	9.62	9.68	100.6
2	9.65	9.89	102.5
3	9.68	9.66	99.8
4	9.69	9.63	99.5
5	9.66	9.58	99.2
6	9.65	9.64	99.8
7	9.63	9.71	100.8
8	8.63	8.51	98.5
9	8.54	8.55	100.1
10	8.58	8.56	99.8

[a]Results obtained from plot of equation (48).

Figure 8. Plot of equation (49) as an example of obtaining the fractional concentrations of the components in a ternary mixture. Data plotted are for sample 1, Table IX.

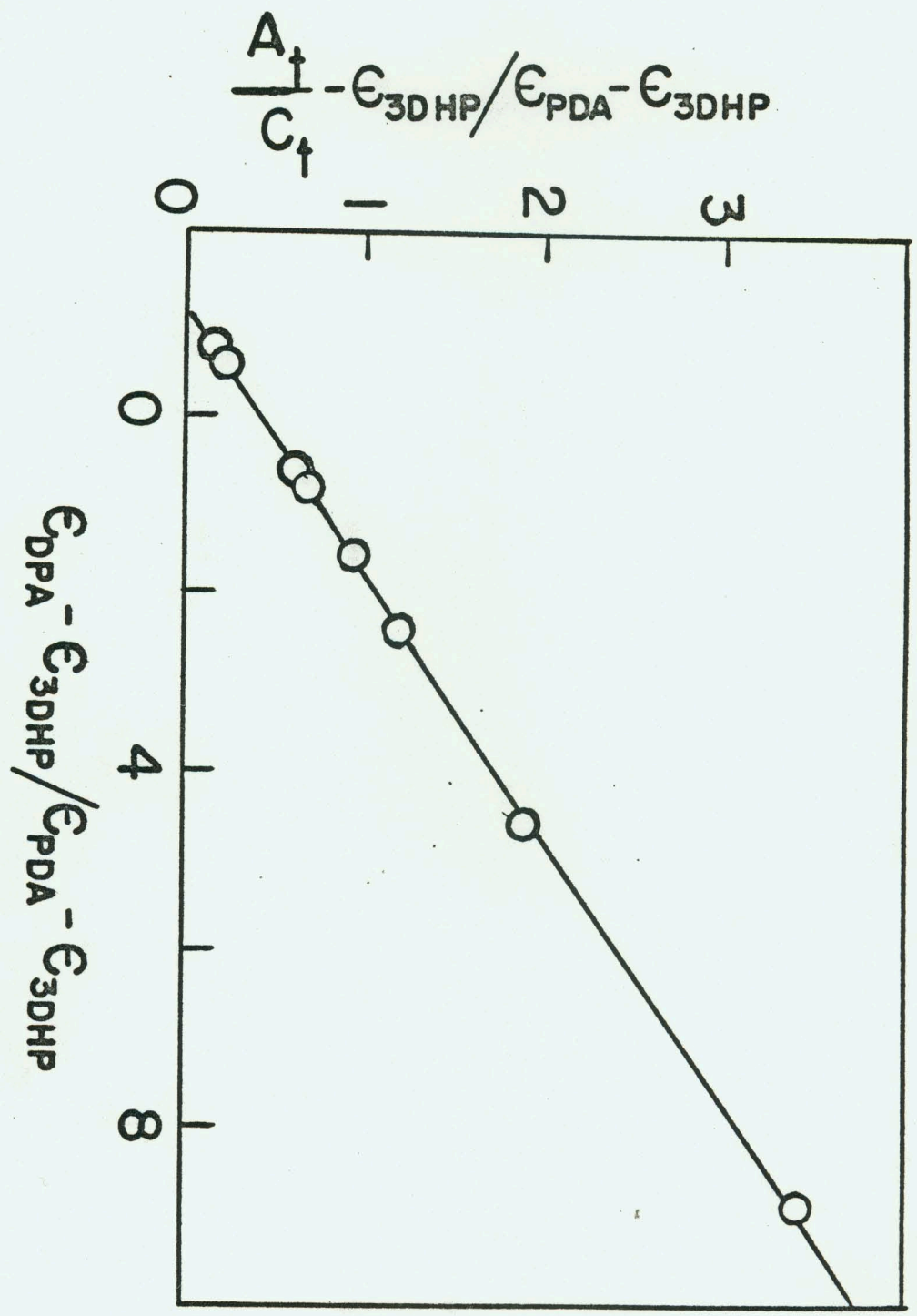

$\frac{A_\uparrow}{C_\uparrow} - \epsilon_{3DHP} / \epsilon_{PDA} - \epsilon_{3DHP}$
0
1
2
3
0
4
8
$\epsilon_{DPA} - \epsilon_{3DHP} / \epsilon_{PDA} - \epsilon_{3DHP}$

Figure 9. Spectra of 2,4-dichlorophenol, 2,5-dichlorophenol and 2,6-dichlorophenol in 95% ethanol.

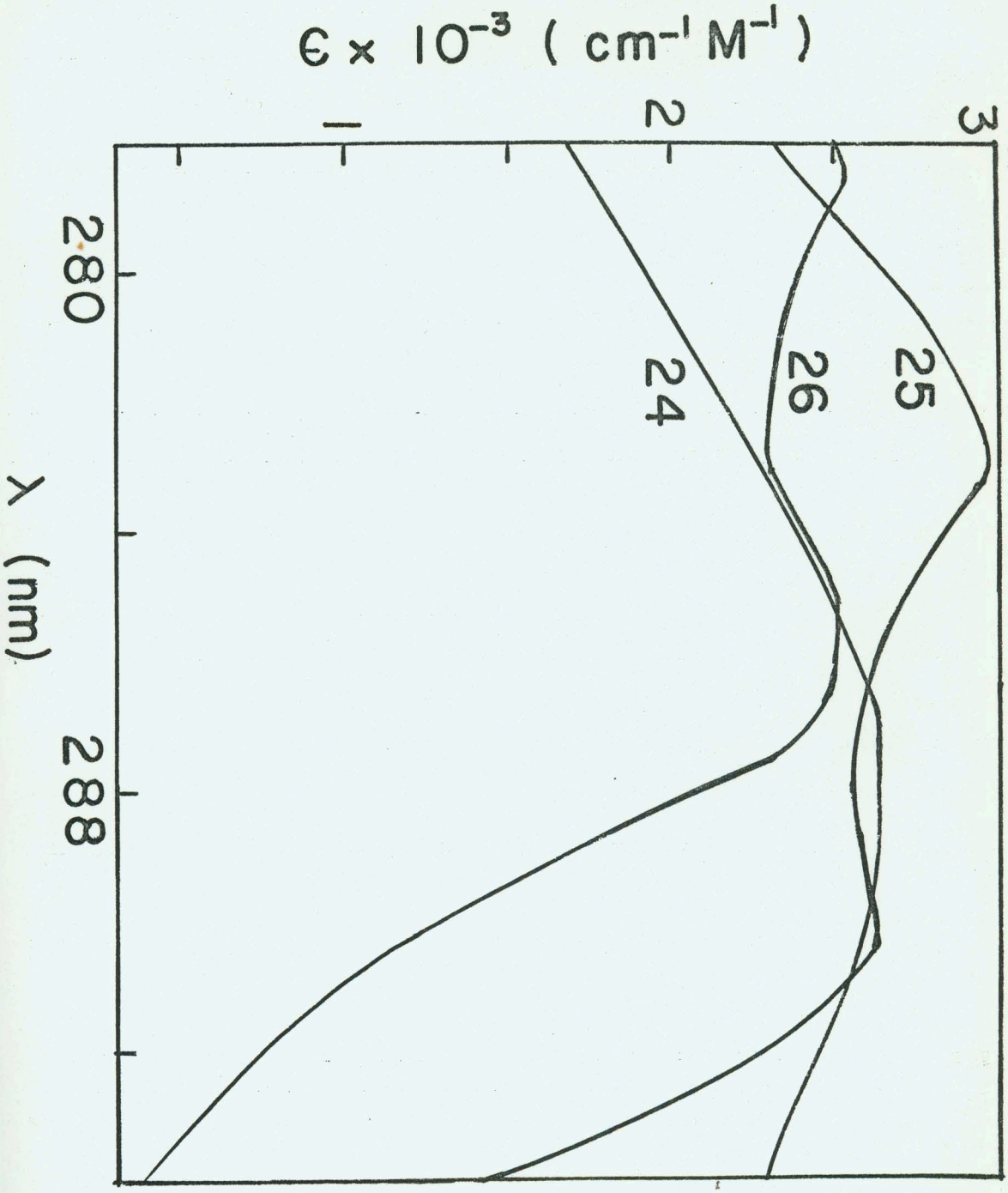

ε × 10⁻³ (cm⁻¹ M⁻¹)
1
2
3
280
288
λ (nm)
24
26
25

Table IX. 2,3-Pyridine dicarboxylic Acid, 2,6-Pyridine dicarboxylic Acid and 2,3-Dihydroxypyridine Mixtures[a]

Sample	Component	Fraction		Concentration x 10^5 M		% Recovery
		Taken	Found	Taken	Found	
1	PDA	0.44	0.44	4.21	4.29	101.2
	DPA	0.34	0.33	3.24	3.19	98.6
	3DHP	0.22	0.23	2.17	2.22	102.6
2	PDA	0.33	0.31	3.16	3.07	97.2
	DPA	0.45	0.47	4.33	4.60	106.2
	3DHP	0.22	0.22	2.17	2.23	102.6
3	PDA	0.22	0.22	2.11	2.13	101.0
	DPA	0.45	0.44	4.33	4.25	98.3
	3DHP	0.33	0.34	3.25	3.28	101.0
4	PDA	0.22	0.22	2.11	2.12	100.4
	DPA	0.33	0.32	3.24	3.08	95.1
	3DHP	0.45	0.46	4.34	4.43	102.1
5	PDA	0.33	0.30	3.16	2.89	93.3
	DPA	0.22	0.25	2.16	2.38	110.0
	3DHP	0.45	0.45	4.34	4.31	99.4
6	PDA	0.33	0.30	3.16	2.89	91.5
	DPA	0.33	0.34	3.24	3.28	101.2
	3DHP	0.34	0.36	3.25	3.47	106.8
7	PDA	0.44	0.44	4.21	4.22	100.3
	DPA	0.22	0.23	2.16	2.18	101.1
	3DHP	0.34	0.33	3.25	3.20	98.5
8	PDA	0.12	0.09	1.05	0.80	72.9
	DPA	0.50	0.54	4.33	4.59	106.1
	3DHP	0.38	0.37	3.25	3.15	96.8
9	PDA	0.49	0.48	4.25	4.10	97.5
	DPA	0.38	0.39	3.24	3.29	101.6
	3DHP	0.13	0.13	1.08	1.02	98.9

Sample	Component	Fraction		Concentration x 10^5 M		% Recovery
		Taken	Found	Taken	Found	
10	PDA	0.37	0.38	3.16	3.21	101.6
	DPA	0.13	0.12	1.08	1.07	99.9
	3DHP	0.51	0.50	4.34	4.28	98.7

[a]Results obtained from plot of equation (49).

291.5 nm were used for the plots in determining the total concentrations by using equation (50)

$$\frac{A_t}{\varepsilon_{24}} = (C_{24} + C_{25}) + \frac{\varepsilon_{26}}{\varepsilon_{24}} C_{26} \qquad (50)$$

Results obtained from such plots are shown in Table X and the mean percentage recovery of the total concentration is (99.7 $\pm$ 0.5)%, N = 8.

Results obtained for the fractional concentrations from the plots using equation (51)

$$\frac{(A_t/C_t) - \varepsilon_{26}}{\varepsilon_{24} - \varepsilon_{26}} = f_{24} + \frac{\varepsilon_{25} - \varepsilon_{26}}{\varepsilon_{24} - \varepsilon_{26}} f_{25} \qquad (51)$$

are shown in Table XI, from statistical analysis, for all concentrations greater than 1.8×10^{-5} M, the overall mean percentage recovery is (99.5 $\pm$ 2.4)%, N = 23; the mean percentage recovery for 2,4-dichlorophenol is (96.7 $\pm$ 4.0)%, N = 8; for 2,5-dichlorophenol (98.0 $\pm$ 2.4)%, N = 7 and for 2,6-dichlorophenol (102.0 $\pm$ 4.0)%, N = 8. The range of wavelength used in the plots for obtaining the fractional concentrations is 270 to 294 nm.

Table X. Total Concentrations of 2,4-Dichlorophenol, 2,5-Dichlorophenol and 2,6-Dichlorophenol Mixtures[a]

	Total Concentration x 10^4 M		
Sample	Taken	Found	% Recovery
1	1.57	1.56	99.8
2	1.57	1.57	100.6
3	1.59	1.57	99.1
4	1.59	1.57	99.1
5	1.58	1.59	100.3
6	1.58	1.57	99.6
7	1.57	1.56	99.1
8	1.40	1.40	99.7

[a]Results obtained from plot of equation (50).

Table XI. 2,4-Dichlorophenol, 2,5-Dichlorophenol and 2,6-Dichlorophenol Mixtures[a]

		Fraction		Concentration x 10^5 M		
Sample	Component	Taken	Found	Taken	Found	% Recovery
1	24	0.43	0.40	6.76	6.25	92.4
	25	0.34	0.35	5.30	5.47	103.2
	26	0.23	0.25	3.60	3.91	108.5
2	24	0.32	0.30	5.07	4.70	92.7
	25	0.45	0.44	7.07	6.90	97.6
	26	0.23	0.26	3.60	4.07	113.2
3	24	0.22	0.22	3.38	3.38	99.9
	25	0.45	0.44	7.07	6.83	96.7
	26	0.34	0.34	5.40	5.50	101.9
4	24	0.22	0.19	3.38	2.99	88.4
	25	0.33	0.33	5.30	5.19	97.9
	26	0.45	0.48	7.20	7.55	104.9
5	24	0.32	0.33	5.07	5.23	103.2
	25	0.22	0.22	3.53	3.49	98.7
	26	0.46	0.45	7.20	7.13	99.1
6	24	0.33	0.31	5.07	4.87	96.0
	25	0.34	0.32	5.30	5.03	94.9
	26	0.34	0.37	5.40	5.81	107.6
7	24	0.43	0.43	6.76	6.69	99.0
	25	0.23	0.22	3.53	3.42	97.0
	26	0.34	0.35	5.40	5.45	100.9
8	24	0.36	0.35	5.07	4.90	96.6
	25	0.13	0.15	1.77	2.10	118.6
	26	0.51	0.50	7.20	7.00	97.3

[a]Results obtained from plot of equation (51).

B. Analysis of Three- and Four-Component Mixtures Using Triangular and Tetrahedral Plots Respectively

2,6-Pyridine dicarboxylic Acid, 2,3-Pyridine dicarboxylic Acid and 2,3-Dihydroxypyridine Analysis

The spectra of the pure components are shown in Fig. 6. Readings at the iso-absorptive points for 2,6-pyridine dicarboxylic acid and 2,3-dihydroxypyridine at 234, 247.8 and 278.3 nm were used for the plots in determining the total concentrations as in page 48. Results obtained from such plots are shown in Table VIII and statistical analysis gives mean percentage recovery of the total concentration as (100.1 $\pm$ 0.8)%, N = 10.

An example of the use of triangular composition diagram in solving for the fractional concentrations in such systems is shown in Fig. 10. Data plotted are for sample 3 of Table XIII. Results obtained from the triangular composition diagram for determining the fractional concentrations are shown in Table XII. For all concentrations greater than 1.1×10^{-5} M, the overall mean percentage recovery is (100.5 $\pm$ 1.6)%, N = 27; the mean percentage recovery for 2,3-pyridine dicarboxylic acid is (100.8 $\pm$ 2.2)%, N = 9; for 2,6-pyridine dicarboxylic acid (100.3 $\pm$ 4.6)%, N = 9; and for 2,3-dihydroxypyridine (100.3 $\pm$ 2.5)%, N = 9. Readings at 260.0 and 282.5 nm were used for the triangular plots.

Figure 10. Full range graphical solution for ternary mixtures using triangular composition diagram. Data plotted are for sample 3 of Table XIII. These are

λ(nm)	ε_{24}	ε_{25}	ε_{26}	ε_{S}
294	2313	1285	378	1203
281	2046	2830	2340	2495

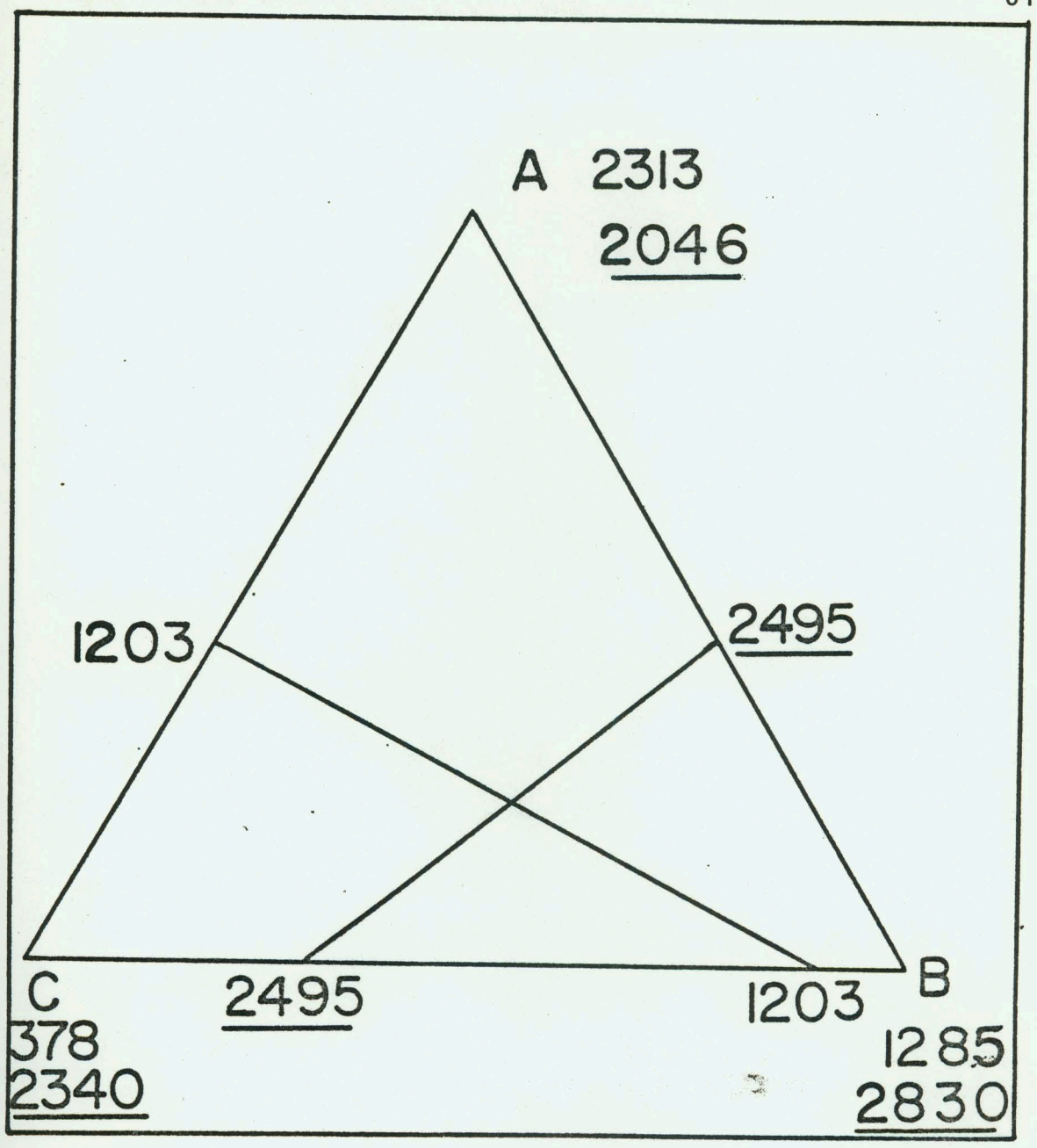

A 2313
2046
1203
2495
C
378
2340
2495
1203
B
1285
2830

Table XII. 2,3-Pyridine dicarboxylic Acid, 2,6-Dicarboxylic Acid and 2,3-Dihydroxypyridine Mixtures

		Fraction		Concentration x 10^5 M		
Sample	Component	Taken	Found	Taken	Found	% Recovery
1	PDA	0.44	0.46	4.21	4.45	105.8
	DPA	0.34	0.30	3.24	2.93	90.4
	3DHP	0.22	0.24	2.17	2.29	105.8
2	PDA	0.33	0.31	3.16	3.07	97.1
	DPA	0.45	0.48	4.33	4.74	109.5
	3DHP	0.22	0.21	2.17	2.09	96.2
3	PDA	0.22	0.22	2.11	2.15	101.9
	DPA	0.45	0.43	4.33	4.10	94.7
	3DHP	0.33	0.35	3.25	3.42	105.1
4	PDA	0.22	0.22	2.11	2.11	100.2
	DPA	0.33	0.32	3.24	3.11	95.9
	3DHP	0.45	0.46	4.34	4.41	101.7
5	PDA	0.33	0.32	3.16	3.10	98.3
	DPA	0.22	0.23	2.16	2.16	100.1
	3DHP	0.45	0.45	4.34	4.31	99.4
6	PDA	0.33	0.32	3.16	3.09	98.0
	DPA	0.33	0.34	3.24	3.32	102.2
	3DHP	0.34	0.34	3.25	3.23	99.3
7	PDA	0.44	0.44	4.21	4.29	101.7
	DPA	0.22	0.23	2.16	2.18	101.0
	3DHP	0.34	0.33	3.25	3.23	99.4
8	PDA	0.12	0.09	1.05	0.72	68.7
	DPA	0.50	0.54	4.33	4.62	106.8
	3DHP	0.38	0.37	3.25	3.16	97.3
9	PDA	0.49	0.49	4.21	4.22	100.3
	DPA	0.38	0.39	3.24	3.31	102.0
	3DHP	0.13	0.12	1.08	1.02	93.8

Sample	Component	Fraction		Concentration x 10^5 M		% Recovery
		Taken	Found	Taken	Found	
10	PDA	0.12	0.38	3.16	3.29	104.1
	DPA	0.50	0.12	1.08	0.99	91.9
	3DHP	0.38	0.50	4.34	4.28	98.7

2,4-Dichlorophenol, 2,5-Dichlorophenol and 2,6-Dichlorophenol Analysis

The spectra of the pure components are shown in Fig. 9. Determination of the total concentrations are as shown on page 56. Results obtained for the fractional concentrations using the triangular composition diagram are shown in Table XIII. For all concentrations greater than 1.8×10^{-5} M, the overall mean percentage recovery is (99.4 ± 1.5)%, N = 23; mean percentage recovery for 2,4-dichlorophenol is (98.9 ± 2.7)%, N = 8; for 2,5-dichlorophenol is (98.7 ± 3.2)%, N = 7; and for 2,6-dichlorophenol is (100.6 ± 3.1)%, N = 8. Readings at 281 and 294 nm were used for the triangular plots.

Summary of Three-Component Mixtures

Table XIV summarizes the results obtained by the proposed methods along with those obtained using the conventional simultaneous equations method.

Benzoic Acid, Salicylic Acid, m-Hydroxybenzoic Acid and p-Hydroxybenzoic Acid Analysis

The spectra of the pure components (except m-hydroxybenzoic acid [mHBA]) are shown in Fig. 11. The total concentration of the individual samples was calculated from the amounts of the components taken in each mixture. Results obtained from the tetrahedral plots used in determining the fractional concentrations are shown in Table XV. Readings

Table XIII. 2,4-Dichlorophenol, 2,5-Dichlorophenol and 2,6-Dichlorophenol Mixtures

		Fraction		Concentration x 10^5 M		
Sample	Component	Taken	Found	Taken	Found	% Recovery
1	24	0.43	0.42	6.76	6.58	97.3
	25	0.34	0.34	5.30	5.24	98.8
	26	0.23	0.24	3.60	3.81	106.0
2	24	0.32	0.31	5.07	4.87	96.1
	25	0.45	0.45	7.07	7.07	100.0
	26	0.23	0.24	3.60	3.73	103.6
3	24	0.22	0.22	3.38	3.43	101.3
	25	0.45	0.44	7.07	6.99	98.9
	26	0.34	0.34	5.40	5.29	98.1
4	24	0.22	0.20	3.38	3.21	94.8
	25	0.33	0.36	5.30	5.58	105.4
	26	0.45	0.44	7.20	6.94	96.4
5	24	0.32	0.34	5.07	5.31	104.7
	25	0.22	0.21	3.53	3.41	96.4
	26	0.46	0.45	7.20	7.13	99.1
6	24	0.33	0.32	5.07	5.04	99.4
	25	0.34	0.32	5.30	5.00	94.3
	26	0.34	0.36	5.40	5.67	105.1
7	24	0.43	0.44	6.76	6.77	100.1
	25	0.23	0.22	3.53	3.44	97.3
	26	0.34	0.34	5.40	5.35	99.2
8	24	0.36	0.35	5.07	4.96	97.7
	25	0.13	0.15	1.77	2.04	115.7
	26	0.51	0.50	7.20	7.00	97.3

Table XIV. Summary of Three-Component Mixtures

Sample	Mean % Recovery		
	By Triangular Plot Solution	From Entire Spectral Range	From Simultaneous Equation[c]
2,3-Pyridine dicarboxylic acid[a]	100.8 ± 2.2	98.2 ± 2.8	97.4 ± 4.2
2,6-Pyridine dicarboxylic acid[a]	100.3 ± 4.6	102.0 ± 3.6	98.6 ± 5.8
2,3-Dihydroxypyridine[a]	100.3 ± 2.5	100.9 ± 2.3	102.5 ± 3.4
2,4-Dichlorophenol[b]	98.9 ± 2.7	96.7 ± 4.0	99.8 ± 2.7
2,5-Dichlorophenol[b]	98.7 ± 3.2	98.0 ± 2.4	97.0 ± 5.3
2,6-Dichlorophenol[b]	100.6 ± 3.1	102.0 ± 4.0	102.6 ± 5.2

[a] Minimum concentration determined is 1.1×10^{-5} M.

[b] Minimum concentration determined is 1.8×10^{-5} M.

[c] Data obtained by conventional simultaneous equation method using readings at three wavelengths.

Figure 11. Spectra of benzoic acid, salicylic acid, and p-hydroxybenzoic acid in approximately 0.02 M sodium hydroxide solution.

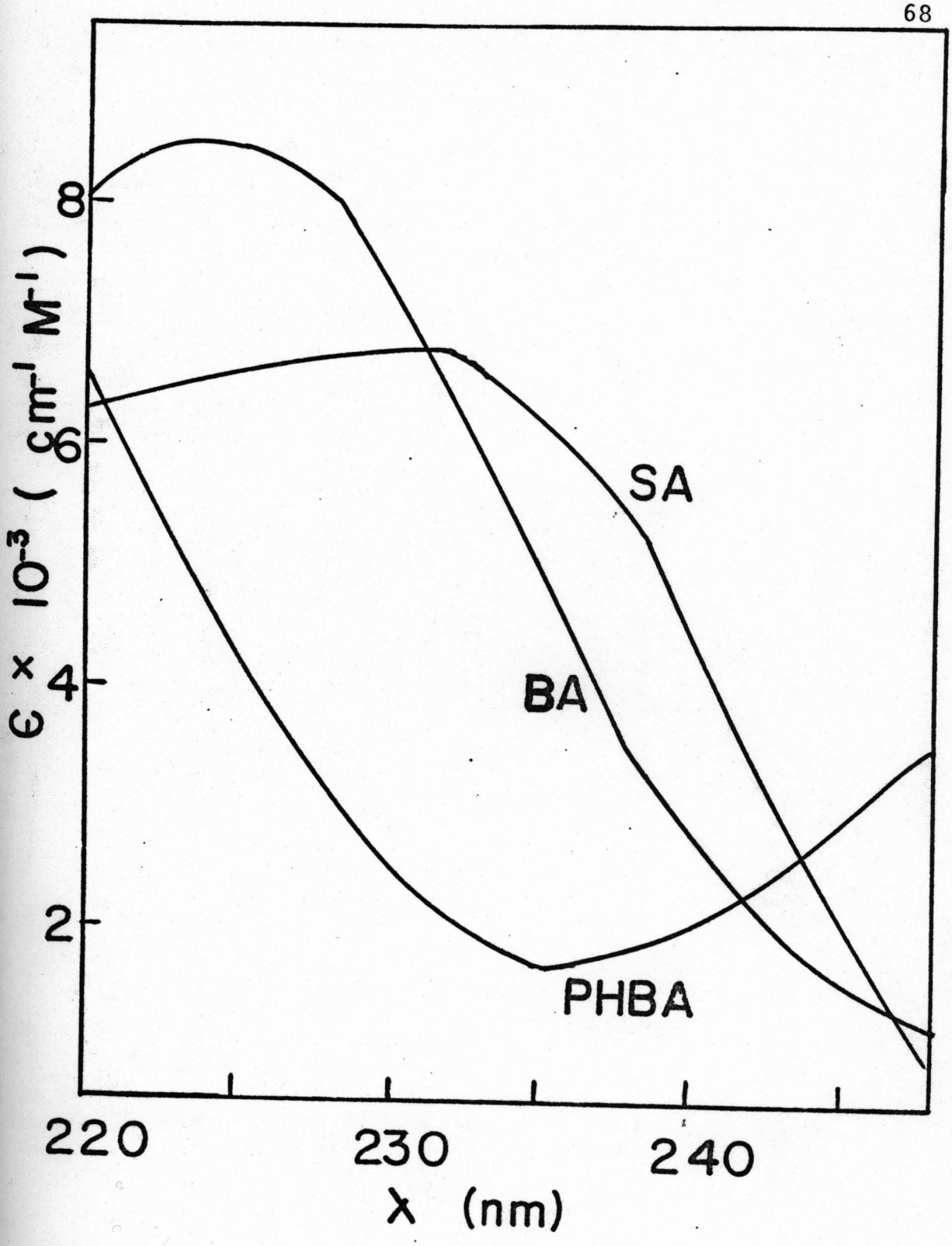

8
6
4
2
ε x 10⁻³ (ℓ m⁻¹ M⁻¹)
SA
BA
PHBA
220
230
240
λ (nm)

Table XV. Benzoic Acid, Salicylic Acid, m-Hydroxybenzoic Acid and p-Hydroxybenzoic Acid Mixtures

Sample	Component	Fraction		Concentration x 10^5 M	
		Taken	Found	Taken	% Recovery
1	BA	0.25	0.24	1.53	94.0
	SA	0.24	0.27	1.43	113.0
	pHBA	0.24	0.22	1.42	93.0
	mHBA	0.27	0.30	1.61	112.0
2	BA	0.36	0.36	2.42	99.4
	SA	0.32	0.31	2.13	97.0
	pHBA	0.21	0.21	1.43	100.4
	mHBA	0.11	0.10	0.77	89.5
3	BA	0.35	0.38	2.42	108.7
	SA	0.21	0.21	1.42	100.0
	pHBA	0.10	0.13	0.72	126.8
	mHBA	0.34	0.30	2.29	90.8
4	BA	0.43	0.46	3.22	106.6
	SA	0.28	0.27	2.13	94.6
	pHBA	0.19	0.20	1.43	104.5
	mHBA	0.10	0.10	0.77	97.7
5	BA	0.21	0.18	1.61	84.3
	SA	0.28	0.31	2.13	109.0
	pHBA	0.10	0.11	0.72	114.5
	mHBA	0.41	0.41	3.06	100.6
6	BA	0.11	0.12	0.81	108.1
	SA	0.29	0.28	2.13	96.9
	pHBA	0.39	0.42	2.86	106.5
	mHBA	0.21	0.19	1.53	88.9
7	BA	0.11	0.11	0.81	106.5
	SA	0.39	0.38	2.84	98.8
	pHBA	0.29	0.30	2.15	101.0
	mHBA	0.21	0.22	1.53	103.6
8	BA	0.42	0.46	3.22	108.3
	SA	0.09	0.05	0.71	56.0
	pHBA	0.19	0.25	1.43	132.4
	mHBA	0.30	0.27	2.29	90.8

Sample	Component	Fraction		Concentration 10^5 M	
		Taken	Found	Taken	% Recovery
9	BA	0.07	0.09	0.48	137.3
	SA	0.30	0.30	2.13	98.2
	pHBA	0.30	0.31	2.15	102.1
	mHBA	0.33	0.32	2.29	98.1

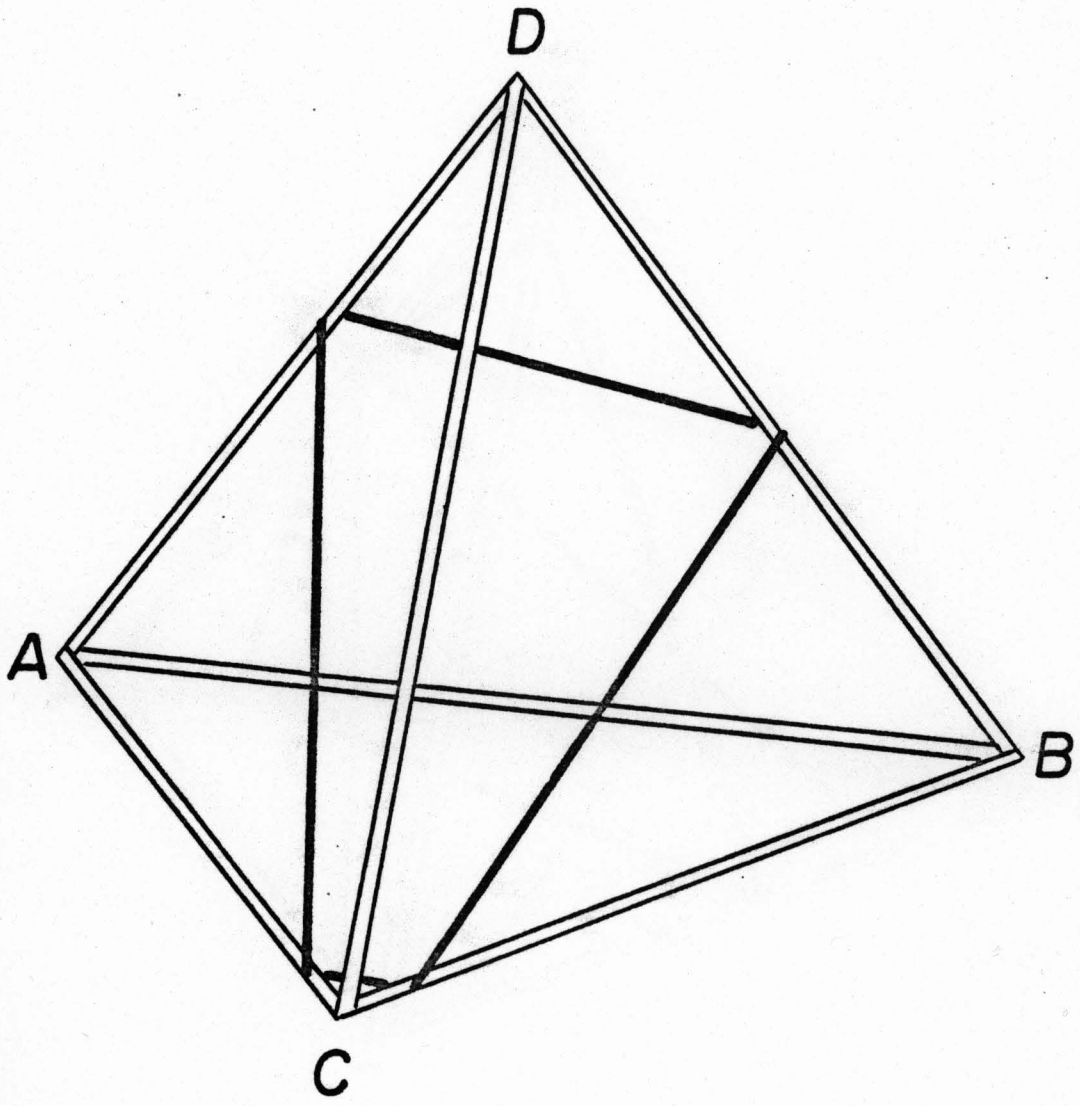
at 225, 235 and 245 nm were used for the tetrahedral plots. The mean percentage recoveries for the components are 100.2, N = 6; 100.9, N = 8; 101.3, N = 6 and 97.8, N = 7 for benzoic acid, salicylic acid, p-hydroxybenzoic acid and m-hydroxybenzoic acid respectively and an overall mean percentage recovery of (100.0 ± 2.9)%, N = 27. An example of a tetrahedral plot using data for sample 9 of Table XVI is shown in Fig. 12. These are

λ(nm)	ε_{PDA}	ε_{DPA}	ε_{3DHP}	ε_{4DHP}	ε_{S}
285	1133	2292	5877	4029	3873
260	2303	3753	1203	2762	2540
230	6178	5614	3751	2222	3948

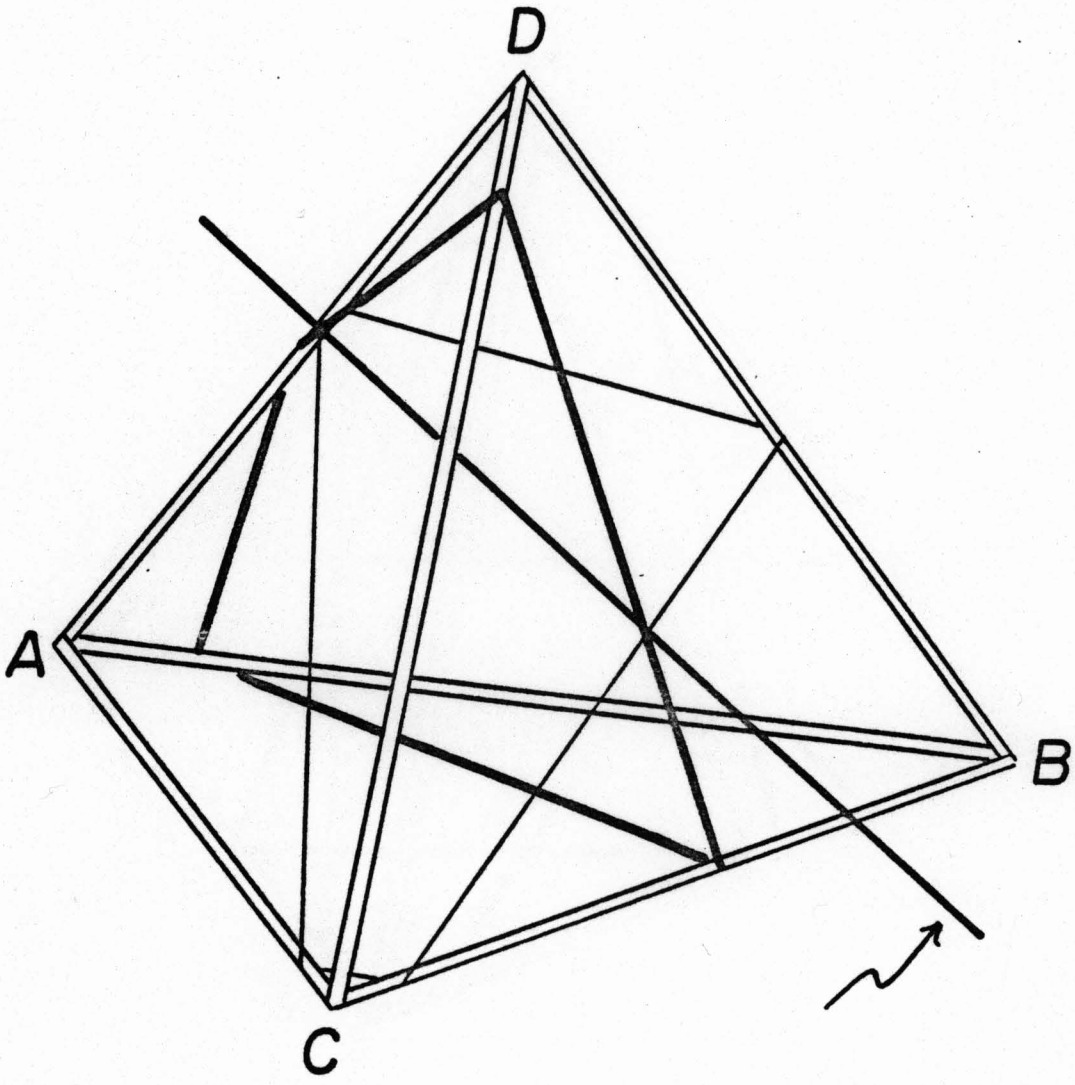
2,3-Pyridine dicarboxylic Acid, 2,6-Pyridine dicarboxylic Acid, 2,3-Dihydroxypyridine, and 2,4-Dihydroxypyridine Analysis

The spectra of the pure components (except 2,4-dihydroxypyridine [4DHP]) are shown in Fig. 6. The total concentration of the individual samples was calculated from the amounts of the components taken in each mixture. Results obtained from the tetrahedral plots used in determining the fractional concentrations are shown in Table XVI. Analysis of the results gives the mean percentage recoveries for 2,3-pyridine dicarboxylic acid as 102.3, N = 7 (rejecting result

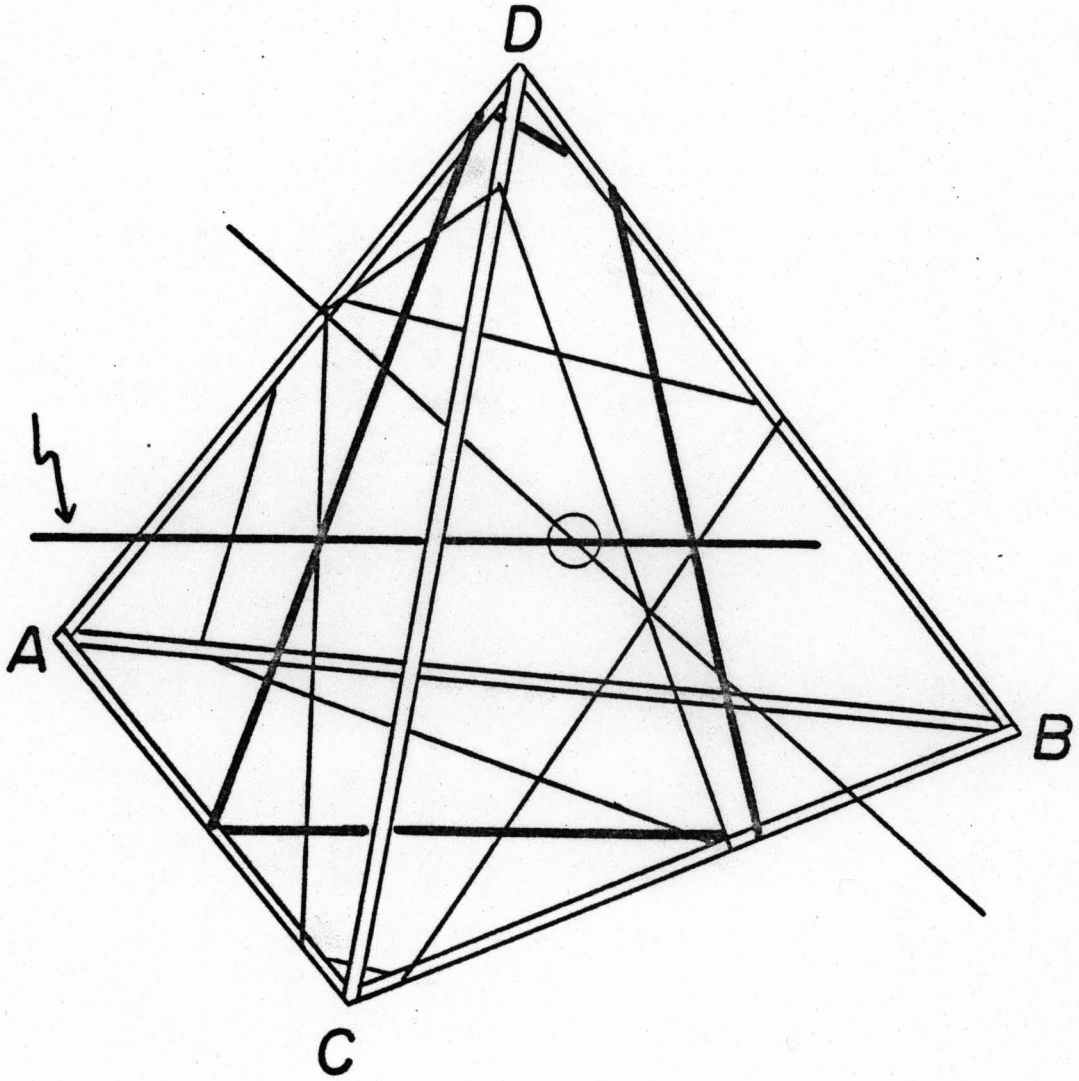
Figure 12. The procedure for doing a tetrahedral plot is shown in Fig. 12 (a, b, c). The data plotted are for sample 9 of Table XVI. The set of molar absorptivities for each of the components are entered at each of the apices of a regular tetrahedron. By linear interpolation, points are marked along the edges corresponding to the "apparent molar absorptivity" of the sample at that wavelength. All such points are connected by a thread which defines a plane. Fig. 12a, shows the plot for the data at the first wavelength. In Fig. 12b, the plot for the data at the second wavelength is shown in dark lines while the light lines correspond to case in Fig. 12a. The points of intersection of these threads are connected by a wire (shown by arrow) which defines a line of intersection of the two planes. The plot using the data for the third wavelength is shown in dark lines in Fig. 12c, while the light lines correspond to case in Fig. 12b. The corresponding points of intersection between this thread and any of the threads for the first or the second wavelength are joined by a wire (shown by arrow). This corresponds to another line where the two planes intersect. The point of intersection of these wires (as shown by the circle) gives the sample composition as it corresponds to a point where the three planes intersect.



(a)



(b)



(c)

Table XVI. 2,3-Pyridine dicarboxylic Acid, 2,6-Pyridine dicarboxylic Acid, 2,3-Dihydroxypyridine and 2,4-Dihydroxypyridine Mixtures

		Fraction		Concentration x 10^5	
Sample	Component	Taken	Found	Taken	% Recovery
1	PDA	0.26	0.27	2.65	103.5
	DPA	0.25	0.20	2.51	82.1
	3DHP	0.24	0.23	2.36	97.1
	4DHP	0.25	0.31	2.56	122.8
2	PDA	0.35	0.37	3.97	106.2
	DPA	0.33	0.29	3.76	87.6
	3DHP	0.21	0.22	2.36	103.7
	4DHP	0.11	0.13	1.28	112.0
3	PDA	0.35	0.36	3.97	103.8
	DPA	0.22	0.21	2.51	95.1
	3DHP	0.10	0.11	1.18	104.7
	4DHP	0.33	0.35	3.83	103.4
4	PDA	0.32	0.37	3.97	115.2
	DPA	0.10	0.05	1.25	49.2
	3DHP	0.48	0.47	5.91	99.2
	4DHP	0.10	0.12	1.28	115.2
5	PDA	0.42	0.42	5.30	101.7
	DPA	0.30	0.29	3.76	97.3
	3DHP	0.18	0.19	2.36	103.2
	4DHP	0.10	0.10	1.28	96.9
6	PDA	0.21	0.22	2.65	107.3
	DPA	0.30	0.28	3.76	94.4
	3DHP	0.09	0.11	1.18	117.2
	4DHP	0.40	0.41	5.11	102.1
7	PDA	0.11	0.10	1.32	95.2
	DPA	0.40	0.40	5.01	98.8
	3DHP	0.29	0.29	3.54	102.5
	4DHP	0.20	0.22	2.56	105.7

Sample	Component	Fraction		Concentration x 10^5 M	
		Taken	Found	Taken	% Recovery
8	PDA	0.42	0.44	5.30	106.5
	DPA	0.10	0.06	1.35	60.6
	3DHP	0.18	0.23	2.46	109.5
	4DHP	0.30	0.30	3.83	100.0
9	PDA	0.07	0.08	0.80	114.3
	DPA	0.31	0.29	3.76	92.6
	3DHP	0.30	0.30	3.54	101.7
	4DHP	0.32	0.34	3.83	106.4

for sample 4); 2,6-pyridine dicarboxylic acid as 94.1, N = 5; 2,3-dihydroxypyridine as 102.4, N = 7; 2,4-dihydroxypyridine as 103.0, N = 4; for concentrations greater than or equal to 1.32×10^{-5} M, 3.76×10^{-5} M, 2.36×10^{-5} M and 3.83×10^{-5} M respectively; and overall mean percentage recovery as (101.0 $\pm$ 2.3)%, N = 23. Readings at 230, 260, and 285 nm were used for the tetrahedral plots.

C. Determination of Acid-Dissociation Constants

Benzoic Acid

The individual concentrations of benzoic acid and benzoate ion [A^-] present at pH = 3.58 and 4.46 were determined from plots of

$$\frac{A_t}{\varepsilon_{BA}} = C_{BA} + \frac{\varepsilon_{A^-}}{\varepsilon_{BA}} C_{A^-} \tag{52}$$

and

$$\frac{A_t}{\varepsilon_{A^-}} = C_{A^-} + \frac{\varepsilon_{BA}}{\varepsilon_{A^-}} C_{BA} \tag{53}$$

These concentrations and pH were used in calculating the pK_a using equations (38) and (41). Results of such determinations are shown in Table XIX. Statistical analysis shows pK_a (corrected) = 4.17 $\pm$ 0.04, N = 4; lit. = 4.19 (44). Readings in the range 220 to 246 nm were used in the plots for calculating the concentrations.

Table XVII. Dissociation Constant of Benzoic Acid

	pK_a'		p_Ka (corrected)	
pH	BA from Slope	BA from Intercept	BA from Slope	BA from Intercept
3.58	4.11	4.10	4.16	4.15
4.46	4.14	4.15	4.19	4.20

Trans-Cinnamic Acid

The individual concentrations of trans-cinnamic acid [TCA] and trans-cinnamate ion [TC^-] at various pH's were obtained from the plots of

$$\frac{A_t}{\varepsilon_{TC^-}} = \frac{\varepsilon_{TCA}}{\varepsilon_{TC^-}} C_{TCA} + C_{TC^-} \qquad (54)$$

at each pH. These concentrations were used in calculating the pK_a using equations (38) and (41). Results of such determinations are shown in Table XX. Statistical analysis of the results shows $pK_a = 4.49 \pm 0.03$, N = 4; lit. = 4.44 (44). Readings in the range 215 to 305 nm were used in the plots for calculating the concentrations.

Table XVIII. Dissociation Constant of Trans-cinnamic Acid

pH	pK_a'	pK_a (corrected)
4.35	4.44	4.49
4.51	4.43	4.48
4.67	4.42	4.47
4.82	4.46	4.51

V. DISCUSSION AND CONCLUSIONS

A. Method Using Entire Spectral Range for Two- and Three-Component Mixtures

As shown by the results obtained in this study, the methods proposed here give accurate and precise results. Thus it is possible to analyze two- and three-component systems by using the entire spectral range. From the results with two-component mixtures, statistical analysis shows no significant difference in determining the concentrations either from the slopes or from the intercepts. However, if one of the concentrations becomes very small, the plot whereby its concentration is found from the slope becomes curved, and neither of the concentrations can be determined. The plot in which it is possible to obtain the higher concentration from the slope and the smaller concentration from the intercept is still linear and both can be determined. In such cases where one of the components is in much smaller concentration, it is better to obtain the smaller concentration from the intercept.

It is better to use the intercept at which the ratio of the molar absorptivities is unity rather than that at which it is zero as zero intercept implies that one of the molar absorptivities is zero or infinite. The intercept at unit ratio gives the total concentration and from knowledge of one of the concentrations from the slope, the other can be determined.

For three-component mixtures, the determination of the total concentration is a prerequisite, and this requires at least two iso-absorptive points for any pair of components. This is not a serious limitation of the method since it is possible to satisfy this requirement in most cases. It might require scanning wide spectral regions for iso-absorptive points. In the plots to obtain fractional concentrations, negative values can be obtained on both axes. This does not arise in two-component analysis. Also, the intercept at which the ratio of the differences in molar absorptivities is zero ($\varepsilon_B - \varepsilon_C / \varepsilon_A - \varepsilon_C = 0$) can be used as this implies equality of the molar absorptivities of the components in the numerator of the right hand side of equation (18).

Non-linearity can occur in the plots for both two- and three-component analysis when data obtained in very weakly absorbing regions of any of the components are used. This is due to large percentage errors in such readings. Thus, it is necessary to obtain data from regions of at least moderate absorption for each of the components. When some of the molar absorptivities of the components are changing rapidly, it is better to scan at low speeds to minimize errors in measuring the absorptions of the samples. It is worth noting that the linear plots are not monotonic functions of wavelengths.

Because many data points are used, the results should be more reliable than those obtained by the simultaneous

equation method. Also, the errors can be minimized using linear least squares regression technique which should be easier to handle mathematically than the fitting methods used by Stenberg et al. (28) and Legget (15) for multicomponent analysis. Hiskey et al. (7) have shown that relative errors are much smaller in multicomponent analysis when relative absorptivities are used instead of absolute absorptivities if the spectra do not overlap completely. Also, Fitzpatrik et al. (24) have shown that the absorbance ratio method (a relative absorptivity method) gave better results than the simultaneous equation method. Since the method proposed here is a relative absorptivity method, one expects it to be capable of better results than the simultaneous equation method. However, the results obtained by the two methods as shown in Tables VII and XIV agree with each other.

Since only two points are required to fix a straight line, data obtained at two wavelengths are enough to obtain the linear plots in determining the concentrations. However, results obtained in this way will not be as reliable as those obtained by using data at many wavelengths, but when time is a factor in the analysis, this can be done.

B. Method Using Triangular and Tetrahedral Plots for Three- and Four-Component Mixtures Respectively

For these solutions, it is necessary to choose the wavelengths for the triangular and tetrahedral plots

correctly. Several desirable features can result from a satisfactory choice of the wavelengths and these can guide the selection. The molar absorptivities must have different relative values at these wavelengths, otherwise the iso-absorptivity planes or lines will not cross. Also, the iso-absorptivity planes or lines should make the largest feasible angle with each other to aid in locating the point of intersection; and the differences in the molar absorptivities at any of the chosen wavelengths should be large relative to the error in measurement of the molar absorptivities. These requirements are equivalent to those for the simultaneous equation method.

The full-range procedure assumes ideal behavior, which is a potential weakness. When non-ideal behavior occurs, mid-range and local solutions could be used. The general procedure for the analysis of an unknown sample should be the preparation of a reference mixture corresponding to full-range compositions and trial of a spectral match with the given sample. If the match is poor, this signifies non-ideality and resort should be made to mid-range and/or local solutions for the spectral match.

It is necessary to note that this method can be looked upon as a means of aiding in the preparation of a reference mixture for the matching process with the sample or as a graphical solution of the simultaneous equations for the system. The results in this report are full-range solutions. From the level of accuracy and precision obtained,

non-ideality was not a problem with these samples. In the kinetic analog of this method, Connors (4) has shown that local, mid-, and full-range solutions do not show much difference if ideal behavior is followed. All the present samples were synthetic, thus their compositions were known beforehand and these agreed well with those determined, as the results indicate. Also, it was not necessary to carry out the spectral match for all the samples, but for cases when they were done (sample 3 of Table XII and sample 2 of Table XV), the match was good. For an unknown sample, the matching process should be carried out unless a graphical solution of the simultaneous equation is anticipated.

The results obtained here agreed well with those obtained using the entire spectral range and with the simultaneous equation method. By comparison with the kinetic methods, the spectrophotometric methods developed in this study have certain advantages because of their independence of kinetic effects such as reaction order and conditions. These methods should be more versatile than the kinetic analogs because the requirement that the components react with a common reagent and yield a common or similar product(s) as required in the kinetic methods is not necessary. Also the excess reagent(s) and other products of reaction can interfere in the kinetic method. However, the limitation is that a component has to absorb, and each component must have a different spectrum. Also, in the four-component analysis, the total concentration has to be determined by an

independent method.

Some of the mixtures analyzed in this study or ones similar to them have been analyzed spectrophotometrically using different methods and work-up procedures. Aspirin and salicylic acid mixtures have been analyzed by the simultaneous equation method (2,45,46), whereas benzocaine and procaine hydrochloride, and procaine hydrochloride and tetracaine hydrochloride mixtures have been analyzed using the absorbance ratio method (20). Sulfonamide mixtures are occasionally analyzed simultaneously by UV-visible spectrophotometry because of the similarity of their spectra (2). Mixtures of sulfadiazine, sulfathiazole and sulfamerazine have been analyzed by simultaneous equation method using data at two iso-absorptive points (48). Also sulfathiazole and sulfapyridine mixtures have been analyzed by absorbance ratio method (20). Different kinds of dichlorophenols as metabolites of dichlorobenzenes in conjunction with other metabolic products have been analyzed by chromatography and UV-detection (47). Benzoic acid and its monohydroxy isomers have been analyzed by the simultaneous equation method. The o-hydroxy isomer was determined as a Fe(III) complex (13). All the results cannot be compared because we have pushed ours to the limit of detection.

C. Conclusions

New graphical, spectrophotometric methods have been developed in this study for the simultaneous analysis of

mixtures. Some of the remarkable features of the methods are their overall simplicity and generality; thus a sample can be analyzed by such methods whether the components are similar or different as long as they have different spectral characteristics. The method using the entire spectral range should be very suitable in handling cases where the spectra are quite similar, as it makes maximum use of the minor differences in their spectra for analysis. Since many plots can be made on the same graph, these methods can be of great aid in routine analyses. This method can be handled by the computer, and by utilizing the capabilities available in it for data treatment, it may be possible to obtain better results and possibly in a shorter time than the ones reported here, and can thus lead to better computer approaches in spectral fitting methods.

There are limitations to the methods. Compared with the simultaneous equation method, the entire spectral range method requires more time when many data points are used. While it is possible to analyze more complex mixtures using simultaneous equation and separation methods, it seems almost impossible at present to do so with the methods developed here.

Lower concentrations can be analyzed using more sensitive responses than the absorption technique. Fluorimetric analysis will be one such method. Because of interference effects (quenching, excimer formation and fluorescence, secondary absorption of emitted light) inherent in such

systems, non-ideality will be a problem. This makes the triangular and tetrahedral solutions more promising, as the mid-range and local solutions can handle such difficulties in these systems.

D. Symbols

A absorbance

a_i activity of component i

B benzocaine

b internal path length of cell

BA benzoic acid

C_i concentration of component i

3DHP 2,3-dihydroxypyridine

4DHP 2,4-dihydroxypyridine

DPA 2,6-pyridine dicarboxylic acid

ε molar absorptivity

f_i fractional concentration of component i

I transmitted radiant power

I_o incident radiant power

I_s ionic strength

K_a acid-dissociation constant

K_a' apparent acid-dissociation constant

M molar

mHBA m-hydroxybenzoic acid

N number of readings used in performing statistical analysis

n number of components in a sample

PDA	2,3-pyridine dicarboxylic acid
pHBA	*p*-hydroxybenzoic acid
SA	salicylic acid
SM	sulfanilamide
ST	sulfathiazole
T	tetracaine hydrochloride
TCA	*trans*-cinnamic acid
TC^-	*trans*-cinnamate ion
$Y_{\pm}$	mean molar activity coefficient
Y_i	activity coefficient of component i
24	2,4-dichlorophenol
25	2,5-dichlorophenol
26	2,6-dichlorophenol

VI. BIBLIOGRAPHY

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