

## SPECTROPHOTOMETRIC ANALYSIS OF THE MIXTURES OF PHOTOSYNTHETIC PIGMENTS

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Our study uses two alternative graphical methods for the spectrophotometric analysis of the pigment mixtures. The first one permits to taken data at multiple wavelengths in order to generate linear plots, from which the concentrations can be determined. It is used for the two and three component mixtures. The second one is a graphical technique for the solution of simultaneous equations. It is carried out on a triangular composition plot for a three-component mixture. Results of these methods are compared with the conventional simultaneous equation method.

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### 1. Introduction

The knowledge on the component concentrations presents a great importance for the interpretation of the experimental data in the study of pigment mixtures. Our work uses alternative graphical methods for the spectrophotometric analysis of these pigment mixtures. We have used these methods in the case of binary and ternary mixtures chlorophyll-a + chlorophyll-b, chlorophyll-a + chlorophyll-b +  $\beta$ -carotene, respectively. The results are compared with those obtained by the conventional simultaneous equation method.

### 2. Theoretical background

Throughout this paper one assumes the validity of Lambert-Beer's law and the additivity of absorbances [1, 2, 7]. The cell path length is 1.0 cm.

#### 2.1 Graphical analysis of data at different wavelengths

For the two-component mixture of chlorophyll-a (Chl-a) and chlorophyll-b (Chl-b), we can write:

$$A_t = \sum_i \epsilon_i \cdot c_i, \quad (1)$$

where  $A_t$  is the total absorbance (i. e. optical density, extinction) of the mixture,  $\epsilon_i$  and  $c_i$  are the molar extinction coefficient and molar concentration of species  $i$ , Chl-a or Chl-b or simply  $a$  or  $b$ , to the same wavelength. For the spectrophotometric analysis,  $\epsilon_a$  and  $\epsilon_b$  must be specific functions of wavelength. Then, from Equation (1) one can write two equivalent specific Equations:

$$\frac{A_t}{\epsilon_a} = c_a + \frac{\epsilon_b}{\epsilon_a} \cdot c_b, \quad (2)$$

or

$$\frac{A_t}{\epsilon_b} = c_b + \frac{\epsilon_a}{\epsilon_b} \cdot c_a. \quad (2')$$

Thus, for Equation (2), a straight-line plot of  $A_t/\epsilon_a$  versus  $\epsilon_b/\epsilon_a$  is performed with data points taken at as many wavelength, as desired. The concentration,  $c_b$ , is obtained from the slope of the straight-line, while  $c_a$  can be evaluated by extrapolation to zero of  $\epsilon_b/\epsilon_a$ . The mixture concentration,  $c_t = c_a + c_b$ , is determined by interpolation at the point where  $\epsilon_b/\epsilon_a = 1$ .

Another way to treat the data points, using equation (2'), is to plot  $A_t/\epsilon_b$  versus  $\epsilon_a/\epsilon_b$ ; then  $c_a$  is found from the slope.

For a three-component mixture of Chl-a (component *a*), Chl-b (component *b*) and  $\beta$ -carotene (component *c*), we can write:

$$A_t = \epsilon_a \cdot c_a + \epsilon_b \cdot c_b + \epsilon_c \cdot c_c. \quad (3)$$

One denote total concentration of the mixture by  $c_t = c_a + c_b + c_c$ , and the fractional compositions by:

$$f_a = \frac{c_a}{c_t}, \quad f_b = \frac{c_b}{c_t} \quad \text{and} \quad f_c = \frac{c_c}{c_t}. \quad (3')$$

With these notations, the Equation (3) becomes:

$$\frac{A_t}{c_t} = \epsilon_a \cdot f_a + \epsilon_b \cdot f_b + \epsilon_c \cdot f_c. \quad (4)$$

The  $A_t/c_t$  ratio can be interpreted as an apparent molar absorptivity of the mixture:  $A_t/c_t = \epsilon_{app}$ . Upon rearrangement, the Equation (4) one obtains:

$$\frac{\epsilon_{app} - \epsilon_c}{\epsilon_a - \epsilon_c} = f_a + \frac{\epsilon_b - \epsilon_c}{\epsilon_a - \epsilon_c} \cdot f_b \quad (5)$$

where we have used the evident condition  $f_a + f_b + f_c = 1$ .

A plot of  $(\epsilon_{app} - \epsilon_c)/(\epsilon_a - \epsilon_c)$  versus  $(\epsilon_b - \epsilon_c)/(\epsilon_a - \epsilon_c)$ , over a range of wavelengths, yields the following fractions:  $f_b$  obtained from the slope and  $f_a$  evaluated by extrapolation of  $(\epsilon_b - \epsilon_c)/(\epsilon_a - \epsilon_c)$  to zero. In order to use the Equation (5), the total concentration,  $c_t$ , of the mixture is need to be known. This is usually accessible through the following analysis. We shall suppose that two components (say *a* and *b*) exhibit at least two iso-absorptive points, when the spectra are compared on a molar basis. Thus, at wavelengths where  $\epsilon_a = \epsilon_b$ , Equation (3) can be written as:

$$\frac{A_t}{\epsilon_a} = (c_a + c_b) + \frac{\epsilon_c}{\epsilon_a} \cdot c_c \quad (6)$$

A plot of  $A_t/\epsilon_a$  versus  $\epsilon_c/\epsilon_a$ , using data only at the iso-absorptive points of component *a* and component *b*, yields the concentration  $c_t$ , as the value of  $A_t/\epsilon_a$  when  $\epsilon_c/\epsilon_a = 1$ . This condition, namely the presence of at least two iso-absorptive points for two of three components, seems to be widely met.

## 2.2 Graphical solutions of simultaneous equations

This technique is analogous to the “method of proportional equations” used in the kinetic analysis of mixtures [6]. It is a rapid graphical solution for the ternary mixtures [4, 5].

In the conventional spectrophotometric analysis of an N-component mixture, measurements are made at N wavelengths, and a set of N simultaneous equations is solved for the N concentrations, after prior determination of N<sup>2</sup> absorptivities (coefficients) on samples of the individual (pure) components.

The three-component problem is conventionally formulated as in Equations (7), (8) and (9), where  $\epsilon_{ij}$  is the molar absorptivity of component *i* (*i* = *a*, *b* or *c*) at wavelength  $\lambda_j$  and  $A_{ij}$  is the total absorbance of the mixture at  $\lambda_j$ :

$$\text{at } \lambda_1: \quad A_{t_1} = \epsilon_{a_1} \cdot c_a + \epsilon_{b_1} \cdot c_b + \epsilon_{c_1} \cdot c_c \quad (7)$$

$$\text{at } \lambda_2: \quad A_{t_2} = \epsilon_{a_2} \cdot c_a + \epsilon_{b_2} \cdot c_b + \epsilon_{c_2} \cdot c_c \quad (8)$$

$$\text{at } \lambda_3: A_{t_3} = \epsilon_{a_3} \cdot c_a + \epsilon_{b_3} \cdot c_b + \epsilon_{c_3} \cdot c_c \quad (9)$$

Dividing these equations by total concentration,  $c_t$ , one obtains a completely determined set of equations:

$$\text{at } \lambda_1: \frac{A_{t_1}}{c_t} = \epsilon_{a_1} \cdot f_a + \epsilon_{b_1} \cdot f_b + \epsilon_{c_1} \cdot f_c \quad (10)$$

$$\text{at } \lambda_2: \frac{A_{t_2}}{c_t} = \epsilon_{a_2} \cdot f_a + \epsilon_{b_2} \cdot f_b + \epsilon_{c_2} \cdot f_c \quad (11)$$

$$1 = f_a + f_b + f_c \quad (12)$$

The additional information on the value of  $c_t$  eliminates the measurements at the third wavelength,  $\lambda_3$ . The Equations (10), (11), (12) can be graphically solved. The total concentration is determined by means of Equation (6), as it was described above. The fractional composition of any ternary solution of  $a$ ,  $b$ ,  $c$  components can be represented by a point on a triangular composition diagram. The corners of the equilateral triangle represent the pure component  $a$ ,  $b$  and  $c$ . The edges represent all possible binary mixtures of  $a$  and  $b$ ,  $a$  and  $c$ , or  $b$  and  $c$  components. The ternary mixtures of  $a$ ,  $b$  and  $c$  components are specified by points in the body of the diagram. The fractional composition of any component is given by the linear fractional distance of the point from the edge opposite the apex corresponding to that component.

The six  $\epsilon_{ij}$  values from Equations (10) and (11) are evaluated on solutions of pure  $a$ ,  $b$  or  $c$  component at wavelengths  $\lambda_1$  and  $\lambda_2$ . These values are introduced in the triangular diagram at the appropriate corners. The quantities  $A_{t1}/c_t = \epsilon_{app1}$  at  $\lambda_1$  and  $A_{t2}/c_t = \epsilon_{app2}$  at  $\lambda_2$  are evaluated for the mixture. At  $\lambda_1$  wavelength, the apparent absorptivity  $\epsilon_{app1}$  must have a magnitude between the members of two of the three pairs  $(\epsilon_{a1}, \epsilon_{b1})$ ,  $(\epsilon_{a1}, \epsilon_{c1})$ , or  $(\epsilon_{b1}, \epsilon_{c1})$ . By linear interpolation, some points on these two sides of the triangle are located corresponding to  $\epsilon_{app1} = A_{t1}/c_t$ . These points are joined by a straight-line. The points on this “tie-line” represent all solutions having this value of  $\epsilon_{app1}$  at wavelength  $\lambda_1$ . In a similar way a second tie-line is constructed for data at wavelength  $\lambda_2$ . The point of intersection of these two tie-lines is the only point that can satisfy Equations (10), (11) and (12). The fractional composition of the mixture is read directly from the graph (see Fig. 7). Suppose that the distance from the point of the tie-line intersection to the edge, which opposites the apex A (component  $a$ ) is  $x_a$ , and the similar distance corresponding to the component  $c$  ( $\beta$ -carotene) is  $x_c$ . The fractional compositions will permit the calculation of the component concentrations:

$$f_a \equiv \frac{c_a}{c_t} = \frac{x_a}{x_a + x_b + x_c} \Rightarrow c_a = f_a \cdot c_t \quad (13)$$

$$f_b \equiv \frac{c_b}{c_t} = \frac{x_b}{x_a + x_b + x_c} \Rightarrow c_b = f_b \cdot c_t \quad (14)$$

$$f_c \equiv \frac{c_c}{c_t} = \frac{x_c}{x_a + x_b + x_c} \Rightarrow c_c = f_c \cdot c_t \quad (15)$$

The assumption of additivity of absorbances is equivalent to the use of linear tie-lines and linear interpolation. In the graphical solution as described (the “full-range” solution), the calibrating measurements are made on pure components  $a$ ,  $b$  and  $c$ , and the nature of the entire plane is defined by its properties at the apices. If the mixtures should become nonideally (nonadditively), the empirical compensation can be made, if one uses for calibrating measurements three solutions whose compositions define a triangle smaller than the entire compositional plane; this smaller area includes the sample mixture. Such “mid-range” and “local” solutions can be used for a Chl- $a$  solution impurified with Chl- $b$  and  $\beta$ -carotene, obtained from a partial purification experiment. An advantage of this graphical technique is that it reveals compositions of calibration standards (after a trial analysis with a full-range solution one can obtain an approximate composition of the sample). For its graphical solution the three-components problem requires measurements at two wavelengths ( $\lambda_1$  and  $\lambda_2$ ), knowledge of the total concentration (from the iso-absorptive points method), and representation on a triangular two-dimensional plot. Extension to the four-component problem requires three wavelengths ( $\lambda_1$ ,  $\lambda_2$ ,  $\lambda_3$ ) and a graphical solution in three-dimensions. The appropriate figure is a regular

tetrahedron, each apex representing a pure component, the edges the six possible binary mixtures, the faces the four ternary mixtures, and points inside the tetrahedron the four-components mixtures.

The technique is a simple, rapid means for solving a set of three or four simultaneous equations. It requires the total concentration  $c_t$  of the mixture which does not appear to be generally accessible. Hence an independent measurement of  $c_t$  must be available.

### 3. Materials and methods

Chl-a and Chl-b, extracted from fresh spinach were chromatographed on the powered sugar column, according to the method of Strain and Svec [9]. The absorption maxima as well as the band intensity ratios, for Chl-a and Chl-b in diethyl ether solutions, satisfied the purity of the commonly used chlorophylls.  $\beta$ -carotene obtained from Merck was used without further purification. Its purity was checked by measuring the band intensity ratios in cyclohexane. High concentrations of  $\beta$ -carotene are obtained without the raise of the solubility difficulties. The spectrum of  $\beta$ -carotene was concentration independent. For all the concentrations and all solutions of Chl-a, Chl-b and  $\beta$ -carotene, there was no apparent deviation from Lambert-Beer's law.

The pure solutions of Chl-a, Chl-b and  $\beta$ -carotene, and the binary mixtures of Chl-a, Chl-b and  $\beta$ -carotene were prepared in ethanol. The concentrations of the pure solutions were:  $c_a^0 = 8.71 \times 10^{-6}$  M (Chl-a),  $c_b^0 = 6.44 \times 10^{-6}$  M (Chl-b), and  $c_c^0 = 2.34 \times 10^{-4}$  M ( $\beta$ -carotene). The absorption spectra, at room temperature, were recorded on a Lambda 2S Perkin-Elmer spectrophotometer. The absorption spectra of pure solutions of Chl-a, Chl-b and  $\beta$ -carotene in ethanol are shown in Fig. 1.

Four samples of binary mixtures (Chl-a and Chl-b in ethanol) were prepared by adding to test-tubes the same volumes of Chl-a solution (0.1 ml) and Chl-b solution (0.2 ml) but different volumes of ethanol: 2.7 ml, 3.7 ml, 4.7 ml and 5.7 ml, respectively.

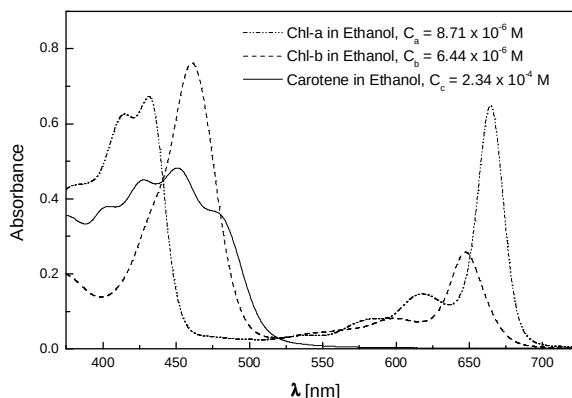


Fig. 1. The absorption spectra in ethanol of Chlorophyll-a, Chlorophyll-b and  $\beta$ -carotene.

The ternary mixtures were prepared in the same manner. For the three samples of ternary mixtures it was added in test-tubes the same volumes of Chl-a solution (0.1 ml), Chl-b solution (0.2 ml) and  $\beta$ -carotene solution (0.7 ml) but different volumes of ethanol: 2 ml, 3 ml and 4 ml, respectively. All these components does not physically and chemically interact between them. The absorption spectra of these four binary mixtures are plotted in Fig. 2, and the absorption spectra of three ternary mixtures are plotted in Fig. 3.

The molar absorption coefficient for Chl-a in ethanol is  $\epsilon_a^{665} = 74400 \text{ M}^{-1}\text{cm}^{-1}$  at  $\lambda = 665 \text{ nm}$ , while for Chl-b in ethanol is  $\epsilon_b^{648} = 40000 \text{ M}^{-1}\text{cm}^{-1}$  at  $\lambda = 648 \text{ nm}$  [9]. Thus, one can find out the concentrations of pure solutions of Chl-a and Chl-b in ethanol and, then, the molar absorptivity at any wavelength. For pure  $\beta$ -carotene solution in ethanol one can compute the concentration from the preparation mode of the stock solution and it can thus find out the molar absorptivity at any wavelength.

It was computed the molar absorptivity for Chl-a, Chl-b and  $\beta$ -carotene in ethanol solutions for a number of 1501 wavelengths in the spectral range from 400 nm to 700 nm with 0.2 nm step, using Microsoft Excel Software. Thus, the statistical parameters for the graphical representation of the Equation (2), used for binary mixtures, and the Equation (5), used for ternary mixtures are very good. All these computed data are based on the experimental data extracted from absorption spectra of pure solutions of Chl-a, Chl-b and  $\beta$ -carotene (see Fig. 1) in ethanol. In the spectral range 400 nm to 700 nm, it was find out five iso-absorptive points for Chl-a and Chl-b, at five wavelengths: 437.8 nm, 577.4 nm, 603.2 nm, 628 nm and 652.8 nm. It was used all these five points to determine the total concentration,  $c_t$ , of the mixtures, using Equation (6).

#### 4. Results and discussions

The analysis based on the Equation (2) can be exemplified by the measurements on binary mixtures of Chl-a and Chl-b in ethanol. The graph from Fig. 4 is represented according to the Equation (2) for these four binary mixtures of Chl-a and Chl-b in ethanol (sample 1, 2, 3 and 4).

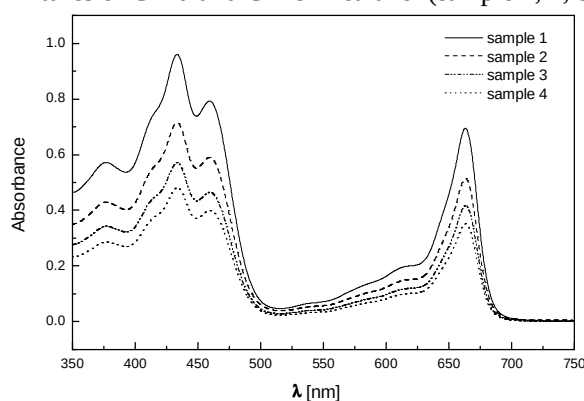


Fig. 2. The absorption spectra of the binary mixtures of Chl-a and Chl-b in ethanol solutions.

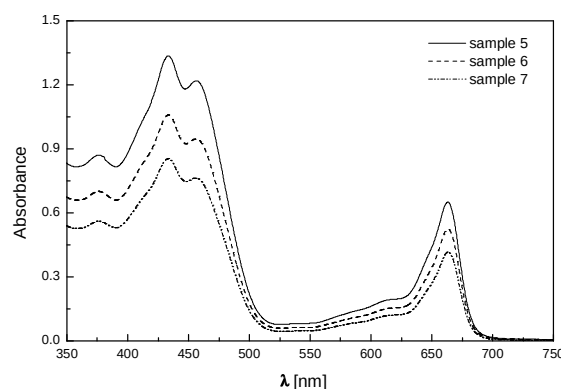


Fig. 3. The absorption spectra of the ternary mixtures of Chl-a, Chl-b and  $\beta$ -carotene in ethanol solutions.

All plots are linear. An interesting feature of the figure is that successive points on the line need not refer to successive wavelength readings. Each component (concentration of Chl-a and concentration of Chl-b) was determined from the slope and the intercept readings. The average error for a particular component appeared to be independent of concentration. To compute the slope,  $c_b$ , and the crossing point,  $c_a$ , from Equation (2) and to estimate the errors, one can use the least-squares-method. Table 1 summarises these results.

The spectrophotometric data for all of these mixtures were also treated by the conventional technique, namely using simultaneous equations set up at two wavelengths.

Table 1. Analytical results on two-component mixtures according to Equation (2).

| Sample | Concentration of Chl-a, $c_a$ (M)  | Concentration of Chl-b, $c_b$ (M)  |
|--------|------------------------------------|------------------------------------|
| 1      | $(7.104 \pm 0.025) \times 10^{-6}$ | $(6.292 \pm 0.004) \times 10^{-6}$ |
| 2      | $(5.794 \pm 0.006) \times 10^{-6}$ | $(4.669 \pm 0.001) \times 10^{-6}$ |
| 3      | $(4.373 \pm 0.010) \times 10^{-6}$ | $(3.708 \pm 0.002) \times 10^{-6}$ |
| 4      | $(3.520 \pm 0.013) \times 10^{-6}$ | $(3.206 \pm 0.002) \times 10^{-6}$ |

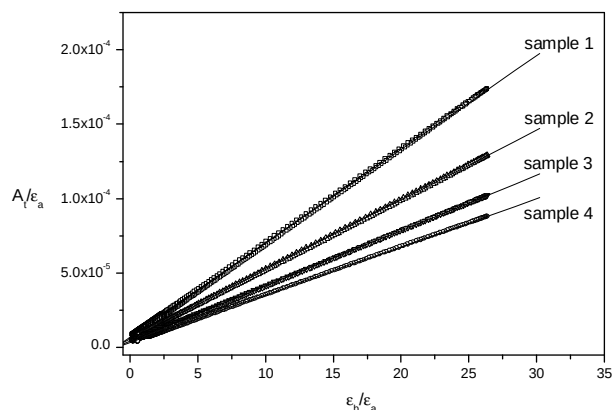


Fig. 4. Plots of the dependence described by Equation (2) for binary mixtures of chlorophyll-a and chlorophyll-b in solutions of ethanol; 1: 0.1 ml Chl-a, 0.2 ml Chl-b in 2.7 ml ethanol, 2: 0.1 ml Chl-a, 0.2 ml Chl-b in 3.7 ml ethanol, 3: 0.1 ml Chl-a, 0.2 ml Chl-b in 4.7 ml ethanol, 4: 0.1 ml Chl-a, 0.2 ml Chl-b in 5.7 ml ethanol.

The errors were greater with the conventional technique than with the new graphical method. This is a reasonable result, because the graphical approach uses more experimental data.

Some three-component mixtures (Chl-a, Chl-b and  $\beta$ -carotene in ethanol) were analysed with Equations (5) and (6). The plot of the Equation (6) applied at the Chl-a – Chl-b iso-absorptive points is shown in Fig. 5. From such plots, the total concentration is given in Table II for all three samples of ternary mixtures.

The plot according to the Equation (5) for three samples (5, 6 and 7) of the ternary mixtures is shown in Fig. 6. The wavelength range was 400 nm to 700 nm.

The individual concentrations for all ternary mixtures were found from the analysis of the plots of Equation (5) using the least-squares-method. The results are shown in Table 3.

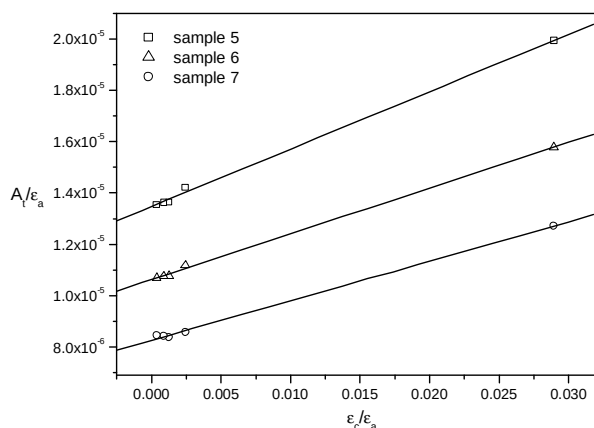


Fig. 5. Plots of Equation (6) for the ternary mixtures of Chl-a, Chl-b and  $\beta$ -carotene in ethanol solutions at the iso-absorptive points at 437.8 nm, 577.4 nm, 603.2 nm, 628 nm and 652.8 nm.

Table 2. Total concentrations on three-component mixtures described by Equation (6).

| Sample | Total concentration, $c_t$ (M)     |
|--------|------------------------------------|
| 1      | $(2.367 \pm 0.054) \times 10^{-4}$ |
| 2      | $(1.886 \pm 0.034) \times 10^{-4}$ |
| 3      | $(1.623 \pm 0.039) \times 10^{-4}$ |

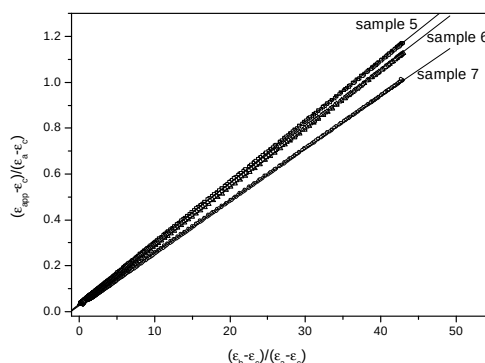
Fig. 6. Plots of Equation (5) for three ternary mixtures of Chl-a, Chl-b and  $\beta$ -carotene in ethanol solutions.

Table 3. Analytical results for three-component mixtures according to Equation (5).

| Sample | Concentration of Chl-a, $c_a$ (M)  | Concentration of Chl-b, $c_b$ (M)  | Concentration of $\beta$ -carotene, $c_c$ (M) |
|--------|------------------------------------|------------------------------------|-----------------------------------------------|
| 1      | $(7.120 \pm 0.162) \times 10^{-6}$ | $(6.299 \pm 0.143) \times 10^{-6}$ | $(2.233 \pm 0.051) \times 10^{-4}$            |
| 2      | $(5.719 \pm 0.104) \times 10^{-6}$ | $(4.835 \pm 0.088) \times 10^{-6}$ | $(1.780 \pm 0.032) \times 10^{-4}$            |
| 3      | $(4.165 \pm 0.101) \times 10^{-6}$ | $(3.706 \pm 0.089) \times 10^{-6}$ | $(1.544 \pm 0.037) \times 10^{-4}$            |

The construction of these plots can be time-consuming, but if many mixtures of the same components must be analysed, the time per analysis becomes very reasonable, because the molar absorptivity functions (e.g., the abscissa function in Equation (5)) are common to all the samples. Only the values of  $A_t$  and  $\epsilon_{app}$  change with the sample.

The triangular composition diagram was applied to the analysis of ternary mixtures of Chl-a, Chl-b and  $\beta$ -carotene for sample 5, 6 and 7. In Table 4 the necessary data for these mixtures are shown. Table 5 gives the concentrations for every component of these mixtures, and Fig. 7 shows how the data are plotted for one of these mixtures, for instance, sample 5. In this figure, Chl-a is designated as A, Chl-b as B and  $\beta$ -carotene as C. The construction of the sample tie-lines by linear interpolation between the apex values is obvious from the figure. The technique was also applied to the other mixtures (samples 6 and 7) using the relations (13), (14) and (15).

Thus, the analytical results are presented in Table 5 obtained by the triangular plotting method, in Table 3 by the linear plotting method and in Table 6 by conventional simultaneous equation method using Equations (7), (8) and (9). The system of Equations (7), (8) and (9) has been solved by the method of Kramer.

Table 4. Data for triangular graphical analysis of ternary mixtures.

|                   | $\lambda$ (nm)                       | 437   | 665   |
|-------------------|--------------------------------------|-------|-------|
| Chlorophyll a     | $\epsilon_a$ ( $M^{-1}cm^{-1}$ )     | 67903 | 74400 |
| Chlorophyll b     | $\epsilon_b$ ( $M^{-1}cm^{-1}$ )     | 63863 | 15997 |
| $\beta$ -carotene | $\epsilon_c$ ( $M^{-1}cm^{-1}$ )     | 1881  | 13    |
| Sample 5          | $\epsilon_{app}$ ( $M^{-1}cm^{-1}$ ) | 5526  | 2700  |
| Sample 6          | $\epsilon_{app}$ ( $M^{-1}cm^{-1}$ ) | 5492  | 2738  |
| Sample 7          | $\epsilon_{app}$ ( $M^{-1}cm^{-1}$ ) | 5149  | 2525  |

Table 5. Results for triangular graphical analysis of a three-component mixtures.

| Sample | Concentration of Chl-a, $c_a$ (M) | Concentration of Chl-b, $c_b$ (M) | Concentration of $\beta$ -carotene, $c_c$ (M) |
|--------|-----------------------------------|-----------------------------------|-----------------------------------------------|
| 1      | $7.29 \times 10^{-6}$             | $6.41 \times 10^{-6}$             | $2.23 \times 10^{-4}$                         |
| 2      | $5.76 \times 10^{-6}$             | $4.81 \times 10^{-6}$             | $1.78 \times 10^{-4}$                         |
| 3      | $4.60 \times 10^{-6}$             | $3.67 \times 10^{-6}$             | $1.54 \times 10^{-4}$                         |

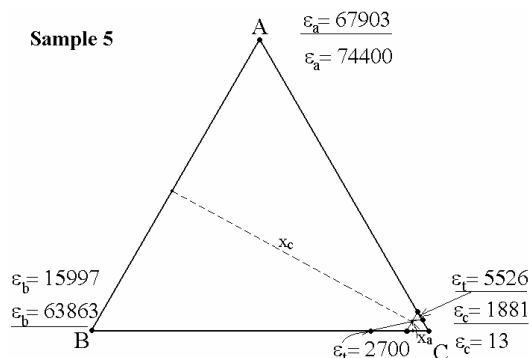
Fig. 7. Graphical analysis of a three-component mixture (Chl-a, Chl-b and  $\beta$ -carotene in ethanol solutions); data are from Table IV; the underlined data refer to  $\lambda = 437$  nm.

Table 6. Concentrations of the ternary mixture compounds using conventional simultaneous equations (7), (8) and (9) at wavelengths: 437 nm, 460 nm and 665 nm.

| Sample | Concentration of Chl-a, $c_a$ (M) | Concentration of Chl-b, $c_b$ (M) | Concentration of $\beta$ -carotene, $c_c$ (M) |
|--------|-----------------------------------|-----------------------------------|-----------------------------------------------|
| 1      | $7.182 \times 10^{-6}$            | $6.358 \times 10^{-6}$            | $2.203 \times 10^{-4}$                        |
| 2      | $5.859 \times 10^{-6}$            | $4.881 \times 10^{-6}$            | $1.733 \times 10^{-4}$                        |
| 3      | $4.660 \times 10^{-6}$            | $3.822 \times 10^{-6}$            | $1.462 \times 10^{-4}$                        |

## 5. Conclusions

A linear plotting method and a rapid graphical solution using the triangular plotting method for two- and three-component pigment mixtures, have been used. The results suggest that there is no clear-cut choice among these three approaches. It seems possible that if the spectra of the three compounds are very similar, the linear plotting method, which uses all the available data, may yield to most reliable analysis, whereas, if the spectra are markedly different, either the triangular plots method or conventional simultaneous equation method may be used.

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