THE DEVELOPMENT OF A SPECTROPHOTOMETRIC METHOD USING FUZZY THEORY

SORANUT KITTIPANYANGAM

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE DEGREE OF DOCTOR OF MATERIAL SCIENCE AND PRODUCTION ENGINEERING

FUKUOKA INSTITUTE OF TECHNOLOGY

ACADEMIC 2019

Contents

Figure contents

Table contents

Abstract

At present, there is no direct concentration measurement method. Therefore, to measure the concentration of solution, an indirect measuring method is employed. After that, the measured value is converted to the concentration. In general, a spectrophotometer, which is a light absorbance measurement device, is used to measure the concentration of solution. The light absorbance measurement is one of the most efficient methods to measure the concentration of solution. To calculate the concentration by using the light absorbance, a spectrophotometric method is usually employed. In a pure solution case, the concentration is calculated by a linear regression analysis based on the Beer-Lambert's law. The linear regression analysis can offer the relationship between the light absorbance and the concentration of solution. In an ideal case, the calculated result is perfectly matched, because of the linear relation between the light absorbance and the concentration of solution. However, in the deviation case from the Beer-Lambert's law, the light absorbance is not proportional to the concentration of solution. Therefore, some errors occur in calculated concentration result. Especially, in the mixture solution case, the error affects the calculated concentration result of all components. For this reason, this research focuses on the reduction of the error by approximating the calculated concentration to an ideal concentration as much as possible. The error is reduced in multi-component case as well as pure solution case.

Until now, many spectrophotometric methods have been proposed for multiple component cases. However, some previous methods depend on a spectrophotometer and the condition of solution. For this reason, the aim of this work is to design the multi-component analysis system that can be used in every spectrophotometer without limited conditions. Concretely, by using fuzzy theory, the proposed method performs the linear interpolation in different ranges of boundary points. In other words, the calculated concentration is expressed as a piecewise-linear function. Thus, the proposed method can reduce errors from existing methods.

To develop a novel multicomponent spectrophotometric method, this research starts from an analysis of the spectrophotometric method of pure solution in section 2, where a light absorbance calculation, a light absorbance measurement, Beer-Lambert's law are discussed. Then, we compare existing multicomponent spectrophotometric methods in the case of 2 components. In section 3, we propose the novel spectrophotometric method using fuzzy theory. The novelty of the proposed method is clarified by explaining the difference between the existing methods and the proposed method. After that, in section 4, we clarify the characteristics of the proposed method by using the computer simulations, and compare the proposed method with existing methods in the ideal case and the deviation of Beer-Lambert's law case. Furthermore, the proposed method is compared with the existing method in experiments by using the light absorbance obtained from a spectrophotometer. Section 5 is the summary and future work of this research.

Keywords: Light Absorbance, UV-spectrophotometer, Beer-Lambert's law, spectrophotometric method, Multi-component analysis, Fuzzy theory

Acknowledgments

I would like to express the deepest appreciation to all those who provided me the possibility to complete this thesis. Firstly, I would like to thank my parent who have supported me. I could not have come this far without their supports. Furthermore, I would like to thank Prof.Dr. Kei Eguchi of material science and production engineering at Fukuoka institute of technology as my advisor. He offered me about the fuzzy theory and the linear interpolation and prepared many devices and many materials for experiments. Therefore, this project has been possible.

Finally, I would like to acknowledge my friends and many staffs of Fukuoka institute of technology providing me with many experiments throughout my years of study.

1. Introduction

1.1. Spectrophotometric method

At present, people use many solutions in everyday life such as drinks, medicine or washing liquid. Each solution has many components and each component has concentration in any level. Therefore, the concentration of solution is an important part to develop the solution. However, there is no direct concentration measurement. The solution is measured by an indirect measuring method. After that, the measured value is converted to the concentration. In chemical laboratory, chemists use spectrophotometers. The spectrophotometer is called that a light absorbance measurement device [1]. It was invented by Arnold O. Beckman in 1940 and it has been developed to the present era. The spectrophotometric method is a method that calculates the concentration by using the light absorbance.

The light absorbance measurement is one way of the effective concentration measurements. It measures the volume of the light intensity transmitting solution to calculate the concentration of solution. The light absorbance is proportional to the concentration of solution following Beer-Lambert's law [2-3]. In the case of a pure solution, the concentration of solution can be calculated by a linear regression analysis. However, the spectrophotometer is very expensive and the chemical faculty has many students. As the result, there is no sufficient fund to purchase the spectrophotometer enough for every student. Therefore, there are many researchers developing the spectrophotometers. The spectrophotometer can be developed by various methods.

The light absorbance depends on the molar absorptivity. This variable is decided by the relationship between solution and the wavelength of light transmitting solution. Thus, some researchers develop the measurement parts. For an example, the monochromator which is a device making the monochromatic light by the visible light [4-5], many light emitted diodes [6-8] or the color light filter for eliminating the disinterest color of light in the visible light [9-10] are developed as light sources. On the other hand, there are researches providing the visible light as the light source and diffracting the light transmitting from solution [11-13]. To measure many colors of light, the photodiode array [11] or many color detectors [14] is utilized as the detector. However, the hand-made spectrophotometer is not similar to the commercial spectrophotometer.

Moreover, in medicine or any solution aspects, there are many components in solution [3,15]. In the multi-component solution, the light absorbance of solution is overlapped by the light absorbance of all components. Thus, the concentration of components cannot be calculated by the light absorbance directly. The multiple spectrophotometric is important in calculating the concentration of the components. Many previous methods have been proposed [16]. Nonetheless, some methods provide the derivative function or the specific case. Many wavelengths of the light source are necessary. Therefore, some methods cannot be used with every spectrophotometer. The target of this research is designed the novel multicomponent spectrophotometric method that can be used with every spectrophotometer.

1.2. Previous multi-component spectrophotometric method

There are many multicomponent spectrophotometric methods. The first multi-component spectrophotometric method is the simultaneous method which provides the mathematics way [17-22]. The next method is the derivative spectrophotometer which utilizes the derivative function to eliminate the light absorbance spectra of the disinterest component at the zero-crossing point [23-28]. The absorbance ratio method employs the light absorbance of standard solution to eliminate the concentration of the disinterest component

[29-31]. The derivative ratio is based on the absorbance ratio and the derivative spectrophotometer which can be used at every wavelength [32-36]. Next is isosbestic point method [37-40] which the molar absorptivity of every component is equal at wavelength of isosbestic point. Q-absorbance ratio method is a term of the absorbance ratio method [41-46]. This technique is modified from the simultaneous equation method. Next technique is absorptivity factor method. This method utilizes the equal of the light absorbance of both wavelengths [47-48]. To analysis solution with more than 3 components, the double divisor spectra derivative method [49-50] and successive ratio-derivative spectra method [16] are utilized. The above-mentioned is one of the multi-component spectrophotometric methods.

In the ideal case, all previous methods are perfect. The concentration results are equal in every method. However, in reality, there is an error of the light absorbance. It is called that deviation of the Beer-Lambert's law. The light absorbance is not proportional to the concentration of solution. The most theory is developed for analyzing the concentration of the components in the ideal case. Therefore, the results of the previous methods have errors in the case of the deviation of the Beer-Lambert's law. For this reason, this proposal focus to reduce an error in the case of deviation of the Beer-Lambert's law.

1.3. Application of multi-component spectrophotometric method

In the present, the multi-component spectrophotometric method has been utilized in many fields, especially in medical term. The multicomponent analysis has been used to calculate the concentration of the component in the medicine, for an example, combination drugs contains Paracetamol and Aspirin [2], Mesalazine and Prednisolone [15], determination of the paracetamol and caffeine in tablet formulation [17], ibuprofen and paracetamol in soft gelatin capsule [18] and etc. In the biochemical term, the multi-component analysis is provided to make the ethidium bromide [36]. In the chemical engineering field, the multi-component spectrophotometric method is utilized to trance metal [37] and measure solution in the suction blister fluid [38].

1.4. Suggestion and contribution in this research

In this thesis, this proposal suggest a novel spectrophotometric method using fuzzy theory. To offer the new method, it is essential to analyze the spectrophotometric method in section 2. It expresses the calculation of the light absorbance, the light absorbance measurement, Beer-Lambert's law. Furthermore, to develop the novel spectrophotometric method, many previous multi-component spectrophotometric methods are analyzed and compared in this section. In the ideal case, the calculated concentration results in every method are the same. Therefore, the calculated results cannot be compared in the ideal case. The comparison is about the linear regression calculation time, the number of input and the specific condition. Moreover, it explains the deviation of Beer-Lambert's law.

Because of the perfection of the calculation in the ideal case, this thesis concentrates on the deviation of Beer-Lambert's law case. In section 3, we suggest the novel spectrophotometric method that reduces errors in the case of the deviation of Beer-Lambert's law. The relationship between the light absorbance and the concentration of solution in the deviation of Beer-Lambert's law is the nonlinear function. The existing method utilizes a linear regression analysis to calculate the concentration as the linear function. Therefore, there are some errors happening. To reduce the errors, a non-linear approximation method is provided. Thus, the many non-linear approximation methods are analyzed in this section. The proposed method provides the linear interpolation to calculate the concentration of solution. The calculation is based on the fuzzy theory. The linear interpolation provides the known concentration solutions as the boundary points [51-52]. The calculated concentration is

explained by the piecewise linear function. Therefore, the number of errors by the calculation of the proposed method is less than the number of errors by the calculation of previous methods. The number of errors is not reduced in only pure solution but also multi-component case. The calculation in the 2-component solution case is similar to the bilinear interpolation [52-54]. Furthermore, the design of the fuzzy set for error reduction is explained in this section.

In section 4, we simulate and compare the spectrophotometric method of the proposed method and the previous methods in the ideal case and the deviation of Beer-Lambert's law case. The comparison is about errors between the calculated concentration and the ideal concentration. Furthermore, we compare the proposed method and the previous methods in a real experiment. The spectrophotometer WPA colour wave CO7500 colorimeter is used to measure the light absorbance.

In section 5, there are discussion, conclusion and future study of this thesis. In the discussion, it expresses the advantages and disadvantages of the proposed methods and the problems of the proposed device. After that, it makes the summary and discusses the future studies.

Contributions of this research are shown as follows:

- Analysis of the spectrophotometric method
- Comparison of the previous multi-component spectrophotometric methods
- Proposal of the spectrophotometric method using fuzzy theory
- Verification of the proposed method in the real experiment

2. Spectrophotometric method analysis

In chemical laboratory, chemists use a spectrophotometer to measure the concentration of solution. The spectrophotometer is a light absorbance measurement device. When the solution is measured by the spectrophotometer, the light absorbance of the solution is converted to the concentration by the spectrophotometric method. This section describes the light absorbance, the relationship between the light absorbance and the concentration following Beer-Lambert's law and the spectrophotometric in pure solution case and the multi-component case. Furthermore, in the real experiment, there are errors in the light absorbance. It is called the deviation of the Beer-Lambert's law. The deviation of the Beer-Lambert's law is explained in this section.

2.1. Concentration calculation by light absorbance

To convert the light absorbance to the concentration of solution, a spectrophotometric method is provided. This subsection explains the light absorbance, the relationship between the light absorbance and the concentration of solution, the spectrophotometric method in pure solution case and the light absorbance of the multi-component case.

2.1.1. Light absorbance

In the environment, the visible light consists of many colors of light. When the light goes through an opaque object, the opaque object absorbs a part of the light. A color of light which is reflected from the object is the color of the object. In the case of the solution, it is the same as the opaque object. The color of the light transmitting solution is the color of the solution [1]. Therefore, each color of the solution absorbs the color of light differently. Furthermore, when the light goes through the solution, the solution does not only absorb one part of the light, it scatters and reflects the light absorbance also shown in figure 2.1. To measure the light absorbance of the solution, the light transmittance is required in calculating. The transmittance (T) is a division between the incident light intensity (I_0) and the transmitting light intensity (I) shown in (2.1). The percentage of the transmittance is calculated by (2.2).

$$T = \frac{I}{I_0} \tag{2.1}$$

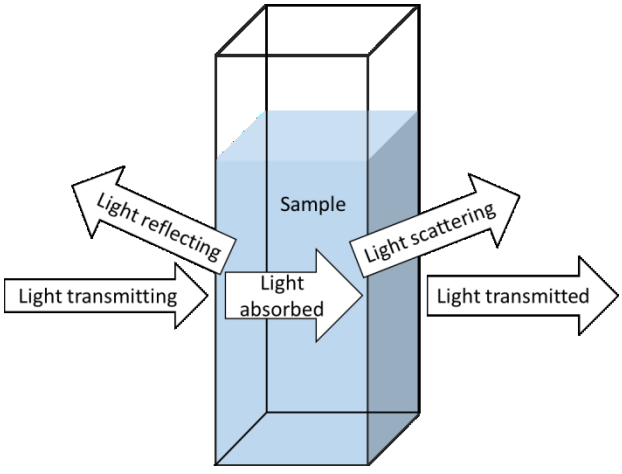


Figure 2.1. Effect when the light goes though the solution.

$$\%T = \frac{I}{I_0} \times 100 \quad (2.2)$$

2.1.2. Lambert's law

Lambert's law is a method explaining the relation between the light absorbance (A) and the path length (l) that the light transmits a solution. The light absorbance is proportional to the path length for parallel beam and monochromatic radiation transmitting a homogeneous medium with the same concentration in (2.3). There are no unit of the light absorbance.

$$A \propto l \quad (2.3)$$

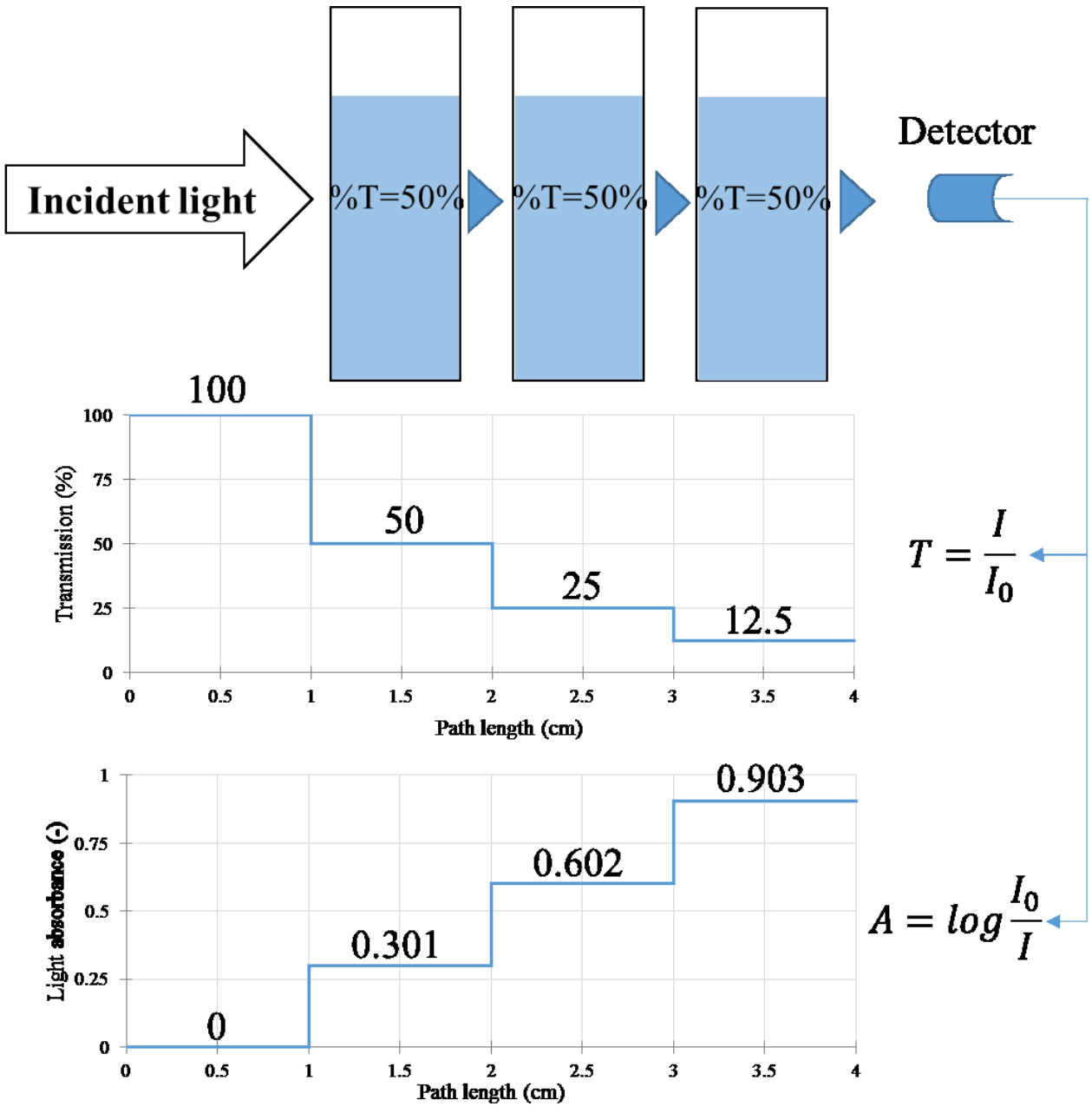


Figure 2.2. Relationship between transmission and absorbance when the path length increases.

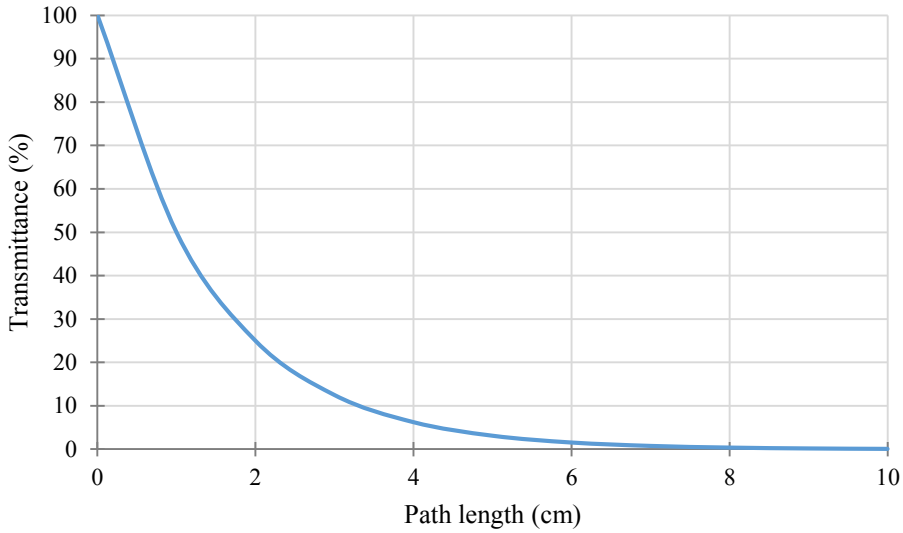


Figure 2.3. Relation between the path length and the transmittance.

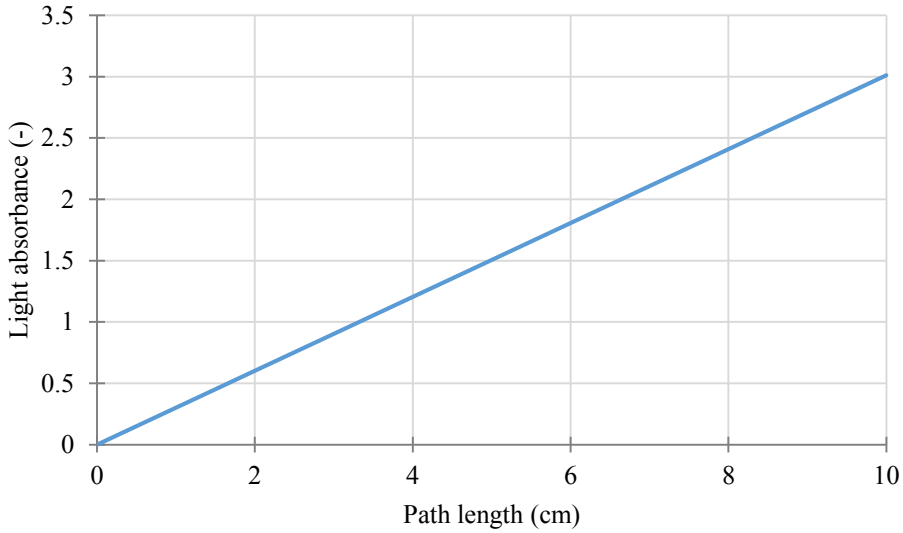


Figure 2.4. Relation between the path length and light absorbance.

Figure 2.2 shows the measurement of solution which each solution has the transmittance 50% (%T). It means that when the light goes through solution, the transmitting light intensity remains 50% of the incident light intensity [48]. When the light goes through each medium, the light intensity decreases. It shows that the transmittance is inverse variation with the path length which light goes through. Therefore, the transmission (T) is inverse variation with the path length (l) which the light goes though shown in figure 2.3. The relationship between the transmission (T) and the path length (l) is exhibited in (2.4). k is a constant value.

$$T = \frac{I_0}{I} = e^{-kl} \tag{2.4}$$

To convert the transmittance which is inverse variation with the path length to the light absorbance which is direct variation with the path length, the logarithm is required. Therefore, the determination of the light

absorbance is the logarithm of the division between the incident light intensity and the transmitting light intensity in (2.5). The light absorbance value does not have the unit. The relation between the path length and light absorbance shown in figure 2.4. It provides the path length which the light transmits as a medium.

$$A = log \frac{I_0}{I} \tag{2.5}$$

2.1.3. Beer's law

Beer's law is a method that explains the relation between the light absorbance and the concentration of solution [1]. The light absorbance (A) is proportional to the concentration of solution (c) for parallel beam and monochromatic radiation transmitting a homogeneous medium with the same path length shown in (2.6). The relationship between the light absorbance and the concentration of solution is illustrated in figure 2.5.

$$A \propto c \tag{2.6}$$

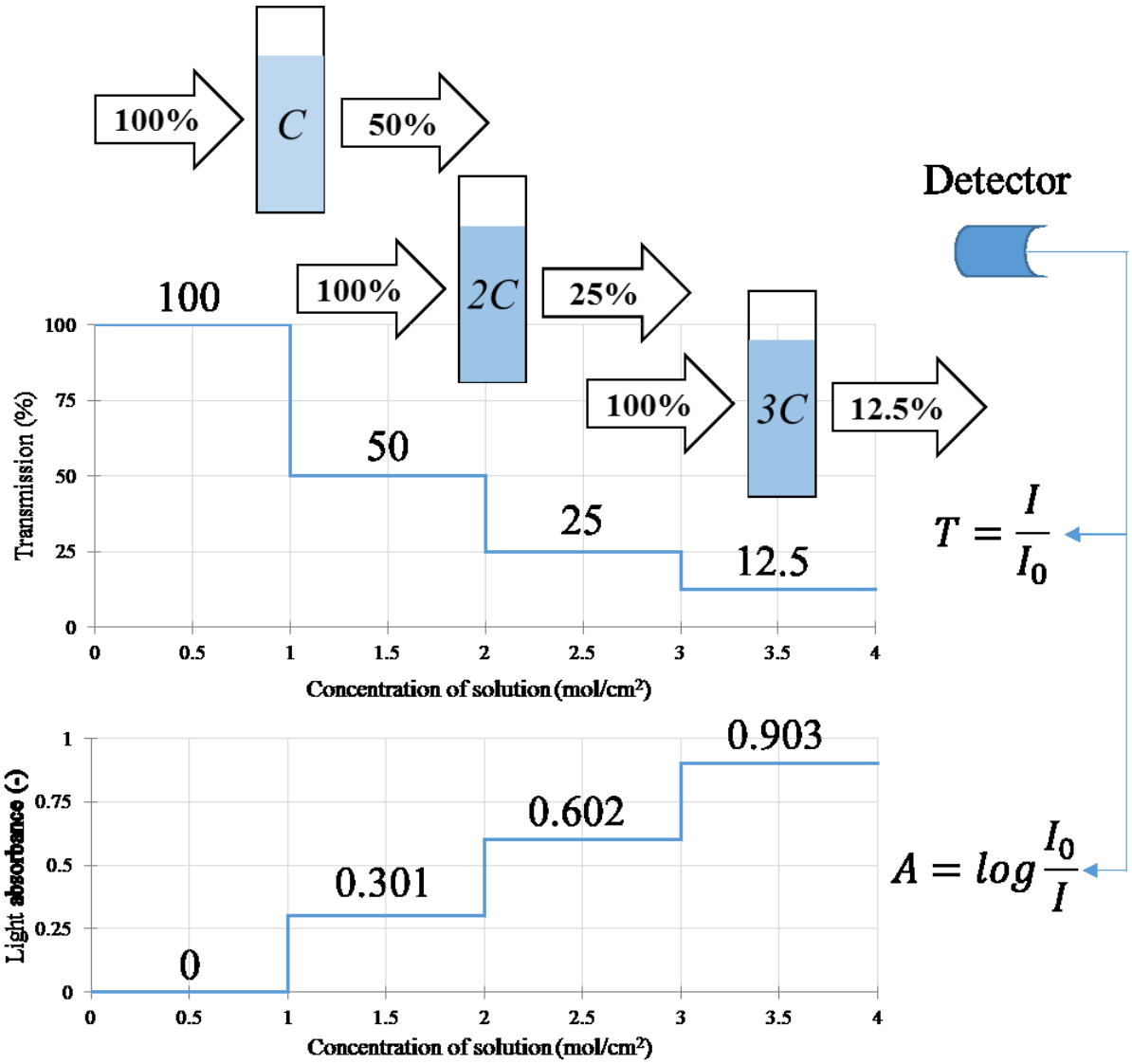


Figure 2.5. Relationship between transmission and absorbance.

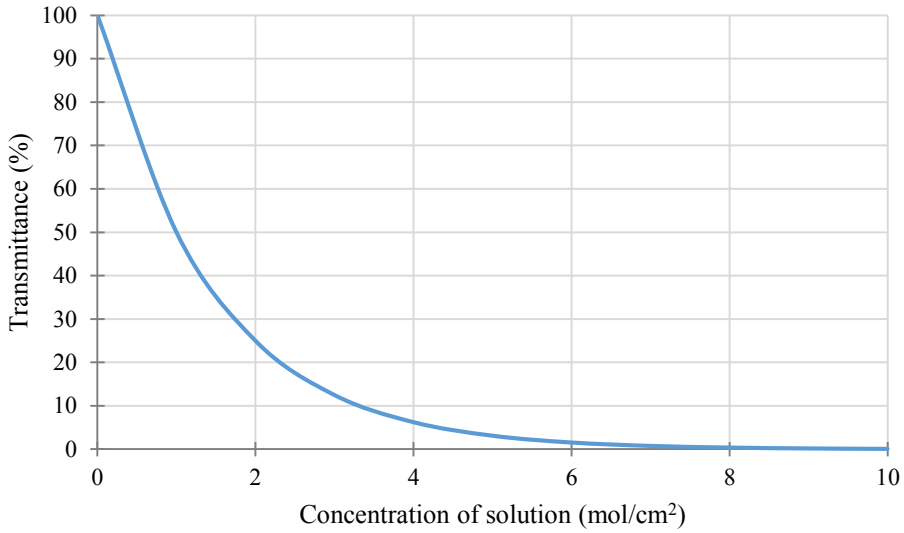


Figure 2.6. Relation between the transmittance and concentration.

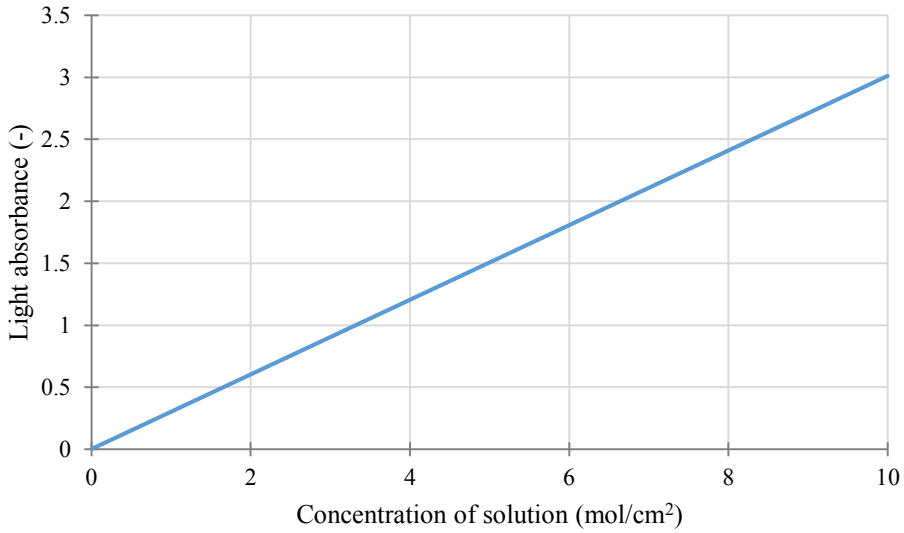


Figure 2.7. Relation between the light absorbance and concentration.

Figure 2.5 shows that when the concentration of the solution, which the transmittance is 50%, increases to 2 times and 3 times, the light transmittance reduces, respectively. The transmittance is inverse variation with the concentration of solution shown in figure 2.6. The relationship between the light absorbance and the concentration calculated by (2.7) is shown in figure 2.7. k is the constant.

$$T = \frac{I_0}{I} = e^{-kc} \tag{2.7}$$

To convert the transmittance which is inverse variation with the concentration of solution in figure 2.6 to the light absorbance which is direct variation with the concentration of solution in figure 2.7, the logarithm is necessary. Therefore, the determination of the light absorbance is the logarithm of the division between the

incident light intensity and the transmitting light intensity in (2.8). It provides the concentration of solution which the light transmits as a medium.

$$A = log \frac{I_0}{I} \tag{2.8}$$

2.1.4. Beer-Lambert's law

A combination of the two laws defines that a light absorbance (A) is proportional to the path length (l) and the concentration of solution (c) for parallel beam and monochromatic radiation transmitting a homogeneous solution [48]. The transmittance (T) is calculated by (2.9).

$$T = \frac{I_0}{I} = e^{-kcl} \tag{2.9}$$

The light absorbance is calculated by taking minus logarithm in (2.9) same as the Beer's law or Lambert's law. Therefore, Beer-Lambert's law equation is shown in (2.10). The molar absorptivity (ε) is a coefficient constant value depending on the color of solution and a wavelength of the light source. In some methods, it provides a as the molar absorptivity. In the measurement, the molar absorptivity and the path length of solution are constant. In an experiment, the path length of solution is 1 cm that does not effect for calculation of the concentration. Therefore, it is eliminated.

$$A = -\log(T) = -\log(\frac{I}{I_0}) = \varepsilon cl \tag{2.10}$$

From the Beer-Lambert's law in (2.10), when the concentration (c) is 0 (solvent), the light absorbance (A) is 0. Therefore, in the light absorbance equation (2.10), the incident light intensity (I_0) can be changed to the light intensity when the light transmits a solvent ($I_{solvent}$). When the solvent is measured, the transmittance is 1. As the result, the light absorbance is 0. Furthermore, the transmitted light intensity (I) is changed to the light transmitting the solution ($I_{solvent}$) in (2.11).

$$A = -\log \frac{I_{solution}}{I_{solvent}} \tag{2.11}$$

However, there is no the light intensity in the electronic circuit. Therefore, a semiconductor, which converts the light intensity into the electrical value, is used such as LDR (Light dependent resistor), photodiode, phototransistor, etc. The properties of these light detectors depend on the light intensity which they detect. For an example, the phototransistor alters the current flowing according to the light intensity. The increase of the electronic current flowing is logistic growth with the light intensity. A resistance of LDR depends on the light intensity that falls upon itself. The resistance varies inversely with the light intensity. Therefore, the voltage of resistor which is connected with the light detector is inverse variation with the concentration of solution. For this reason, the voltage value ($V_{solution}, V_{solvent}$) can replace the light intensity ($I_{solution}, I_{solvent}$) in (2.12) [56].

$$A = -\log \frac{V_{solution}}{V_{solvent}} \tag{2.12}$$

However, the light absorbance does not vary directly with the concentration of solution in some cases. Therefore, to reduce the number of errors of the measurement, the voltage when there is no light falling on the photo detector (V_0) is declined as shown in (2.13) [56].

$$A = -\log \frac{V_{solution} - V_0}{V_{solvent} - V_0} \tag{2.13}$$

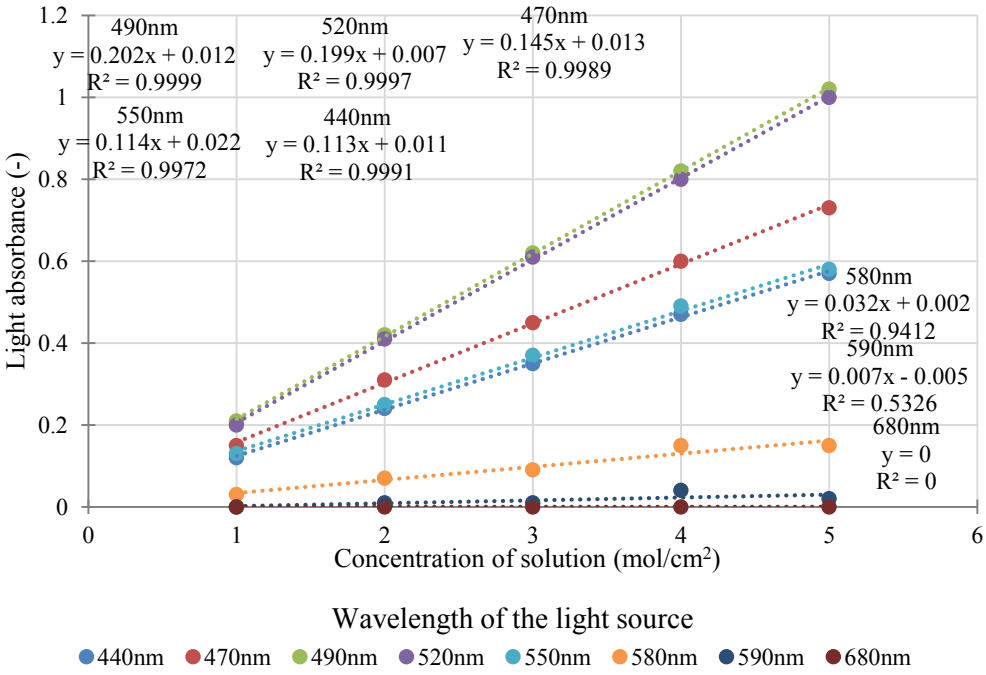


Figure 2.8. Light absorbance of red solution by many wavelengths of the light source.

From the Beer-Lambert's law in (2.10), the light absorbance depends on the molar absorptivity (ε), the concentration of solution (c) and the path length of the light (l). In the measurement, the path length is constant value and the concentration is unknown value. Therefore, the light absorbance value depends on the molar absorptivity. Figure 2.8 shows the light absorbance results of the red solution in many cases of the wavelength of the light source by Biochrom WPA CO7500 Colorimeter. The alteration of the wavelength of a light source changes the molar absorptivity. There are many cases which can observe the rising of the light absorbance or cannot observe. To calculate the concentration of solution easily and observe the growth light absorbance simply, the case of the highest light absorbance (the highest molar absorptivity) is provided to make the linear regression equation.

2.1.5. Coefficient of determination

Although the concentration calculation by the highest molar absorptivity is the best, it does not mean that the light absorbance of other wavelengths cannot be used to calculate the concentration of solution. The coefficient of determination is a value that indicates how well data fit with a statistic model. In the light absorbance measurement, it is used to check how well of the relationship between the light absorbance and the concentration of solution as Beer-Lambert's law. The coefficient of determination is calculated by the square of the correlation coefficient (R^2) in (2.14) [57-58]. x is the concentration of solution and y is the light absorbance. $\bar{x}$ is an average of the concentration of solution and $\bar{y}$ is an average of the light absorbance. N is the number of the data (the number of the known concentration solutions).

$$R^2 = \left\{ \frac{\sum_{i=1}^{N}(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum_{i=1}^{N}(x_i-\bar{x})^2 \sum_{i=1}^{N}(y_i-\bar{y})^2}} \right\}^2 \tag{2.14}$$

A range of the coefficient of determination is from 0 to 1. An ideal value is 1 when *y* varied directly with *x* perfectly shown in figure 2.9. It means that if the coefficient of determination is approximate to 1, the relation between the light absorbance and the concentration of solution is followed in Beer-Lambert's law. To calculate the concentration of solution efficiently, the wavelength that the coefficient of determination is approximate to 1 is utilized.

2.1.6. Linear regression analysis

To calculate the concentration of solution from the light absorbance, a spectrophotometric method is used. In a pure solution case, the spectrophotometric method provides a linear regression analysis. The linear regression analysis provides many known concentration solutions to calculate a linear function that is a relationship between the light absorbance and concentration following Beer-Lambert's law shown in figure 2.9. The linear function is shown in (2.15) [59].

$$Y=a+bX \tag{2.15}$$

When the linear regression equation in (2.15) is compared with the Beer-Lambert's law in (2.10), Y is the light absorbance (A). X is the concentration of solution (c). b is the molar absorptivity (ε) and the path length (l) which is a constant value. a is the light absorbance when the concentration is 0. In an ideal term, a is 0 following Beer's laws equation in (2.10). However, in the measurement, there are some errors. Therefore, a is almost zero. b is a slope of the graph which is calculated by (2.16). a is the light absorbance when the concentration is 0. It is calculated by (2.17). N is the number of the data (the number of the known concentration solutions).

$$b = \frac{\sum_{i=0}^{N} X_i Y_i - \frac{\sum_{i=0}^{N} X_i \sum_{i=0}^{N} Y_i}{N}}{(\sum_{i=0}^{N} X_n^2) - N(\bar{X})^2} \tag{2.16}$$

$$a = \bar{Y} - b\bar{X} \tag{2.17}$$

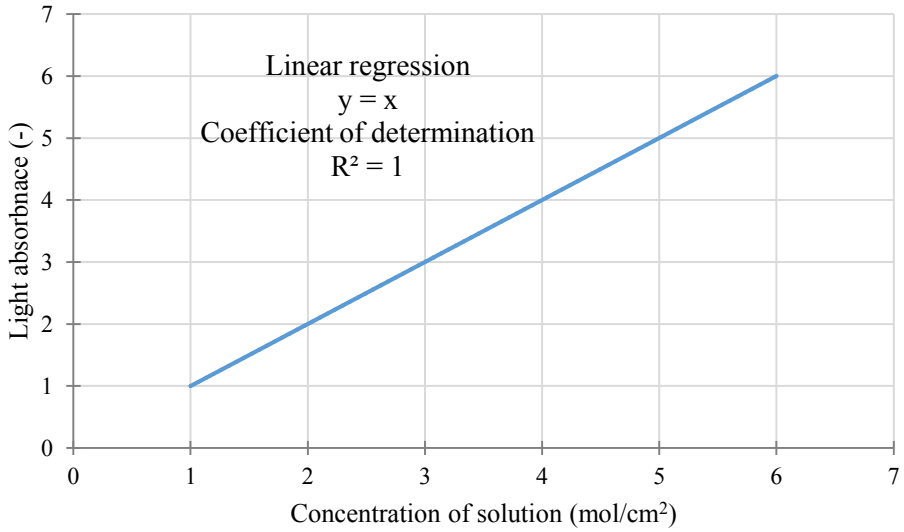


Figure 2.9. Ideal light absorbance.

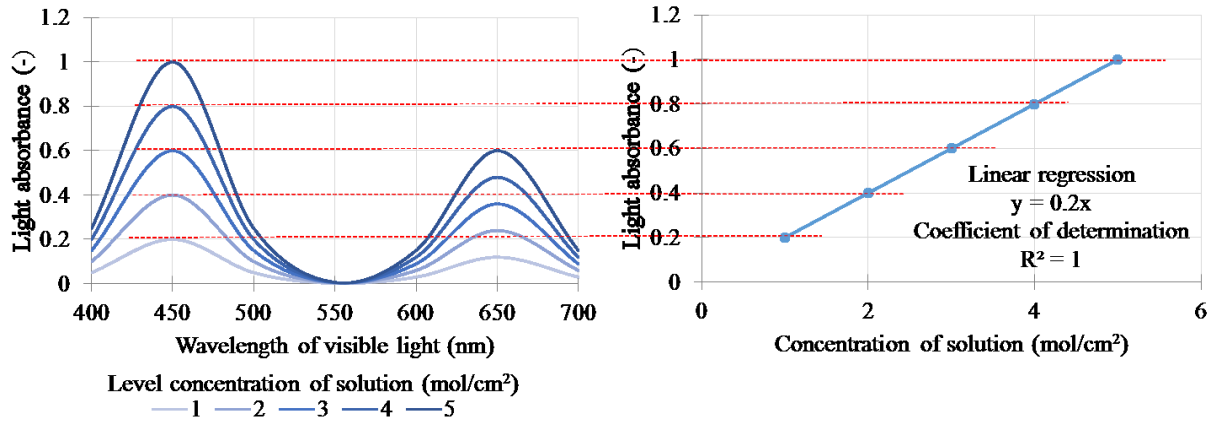


Figure 2.10. Design of linear function that is the relation between the concentration and the light absorbance.

To calculate the concentration of the solution, the light absorbance of the solution is measured by a spectrophotometer. The left-hand graph of figure 2.10 illustrates examples of the light absorbance in the range of the visible spectrum wavelength about 380-740 nm. The spectrophotometer provides the light in the visible spectrum wavelength as a light source to measure the light absorbance. To calculate the concentration clearly, the linear regression analysis provides the light absorbance of many concentrations to calculate the linear function in the right-hand graph of figure 2.10. The light absorbance is provided is the highest light absorbance in the range of the visible light at 450 nm of wavelength. The linear function is the relation between the concentration of solution and the light absorbance in (2.15). Therefore, when the light absorbance is measured, the concentration is calculated by the linear function calculated by the linear regression analysis in the case of the pure solution [1,48].

2.1.7. Multicomponent

However, in the case of the multi-component solution, the light absorbance of the mixture solution (A_M) is the sum of the light absorbances of each component shown in (2.18). Therefore, the concentration of the components cannot be calculated by the linear regression analysis directly. To analyze the concentration of the components, there must be use of any methods shown in section 2.2.

$$A_M = A_x + A_y + \cdots = \varepsilon_x c_x l + \varepsilon_y c_y l + \cdots \tag{2.18}$$

2.2. Comparison of the multicomponent analysis

In this section, the explanation about the previous multi-component spectrophotometric techniques is presented. There are the calculation, advantages, and disadvantages of each method. Each technique has a different calculation process. After that, they are compared by the number of the inputs, the linear regression calculation time and the specific condition.

2.2.1. Simultaneous equation method

The simultaneous equation method is a simple mathematic to calculate the concentration of solution [17-22]. This technique requires the number of the light absorbance equation in any wavelengths equal to the number of the components in the mixture solution. In the case of the 2 components, the light absorbance is the sum of the light absorbance of 2 components. The light absorbance (A_{M_1}, A_{M_2}) is measured by wavelength 1 and wavelength 2 shown in (2.19) and (2.20), respectively. The components in the mixture consist of the substance *x* and substance *y*. c_x and c_y are the concentration of solution of substance *x* and substance *y,* respectively. a_{x_1} and a_{x_2} are the molar absorptivity between the substance *x* and wavelength 1 and wavelength 2, respectively. a_{y_1} and a_{y_2} are the molar absorptivity between the substance *y* and wavelength 1 and wavelength 2, respectively.

$$A_{M_1} = a_{x_1}c_x + a_{y_1}c_y \tag{2.19}$$

$$A_{M_2} = a_{x_2}c_x + a_{y_2}c_y \tag{2.20}$$

To calculate the concentration of each component, the equations (2.19) and (2.20) are rewritten by (2.21) and (2.22), respectively.

$$c_x = \frac{A_{M_2} - a_{y_2}c_y}{a_{x_2}} \tag{2.21}$$

$$c_y = \frac{A_{M_1} - a_{x_1}c_x}{a_{y_1}} \tag{2.22}$$

The simultaneous equation technique eliminates the disinterest component variable from the light absorbance equation by substituting. The concentration of the substance *x* (c_x) in (2.21) are substituted into (2.20). The concentration of the substance *y* (c_y) in (2.22) is substituted into (2.19). The rewritten equation is obtained in (2.23) and (2.24) that are the concentration of the substance *x* and substance *y*, respectively.

$$c_x = \frac{a_{y_1}A_{M_2} - a_{y_2}A_{M_1}}{a_{x_2}a_{y_1} - a_{y_2}a_{x_1}} \tag{2.23}$$

$$c_y = \frac{a_{x_2}A_{M_1} - a_{x_1}A_{M_2}}{a_{x_2}a_{y_1} - a_{y_2}a_{x_1}} \tag{2.24}$$

The process of the simultaneous equation method is shown in figure 2.11. To calculate the concentration, the molar absorptivity is calculated by the slope of the linear regression equation. It is calculated by the known concentration data in (2.16). *X* is the concentration of the component and *Y* is the light absorbance of pure solution. After that, the concentration calculation provides the light absorbance measured by 2 wavelengths and the molar absorptivity calculated by the linear regression analysis in (2.23) and (2.24).

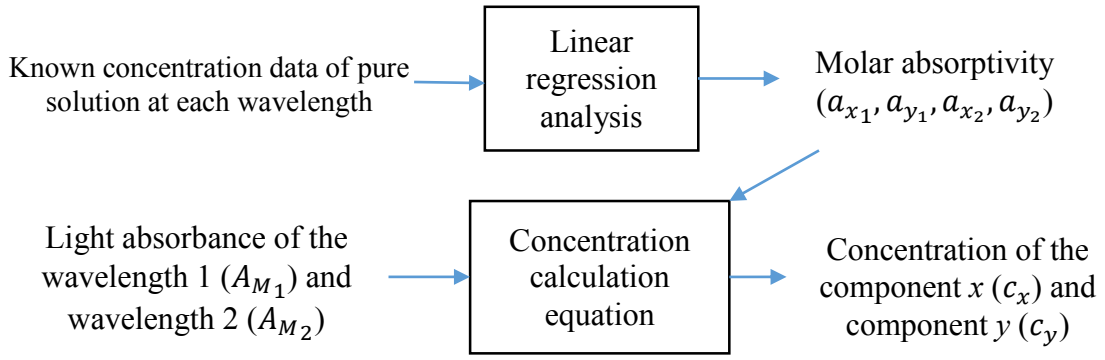


Figure 2.11. Process of the simultaneous equation method.

2.2.2. Derivative spectrophotometry

This method relates to the alternation of the absorption spectra or the zero-order spectrum to the first-order derivative spectrum or the high order [23-28,60]. The width of the high-order wave is narrower than the width of the low-order wave. Therefore, the high order spectrum has more detail than the low order spectrum. Figure 2.12 illustrates examples of the light absorbance of the component *x*, component *y*, and mixture between component *x* and *y* that the spectrum is overlapped by the light absorbance of component *x* and component *y*. The light absorbance of the mixture is calculated by (2.25). ε_x and ε_y are the molar absorptivity of component *x* and component *y*.

$$A_M = \varepsilon_x c_x l + \varepsilon_y c_y l \tag{2.25}$$

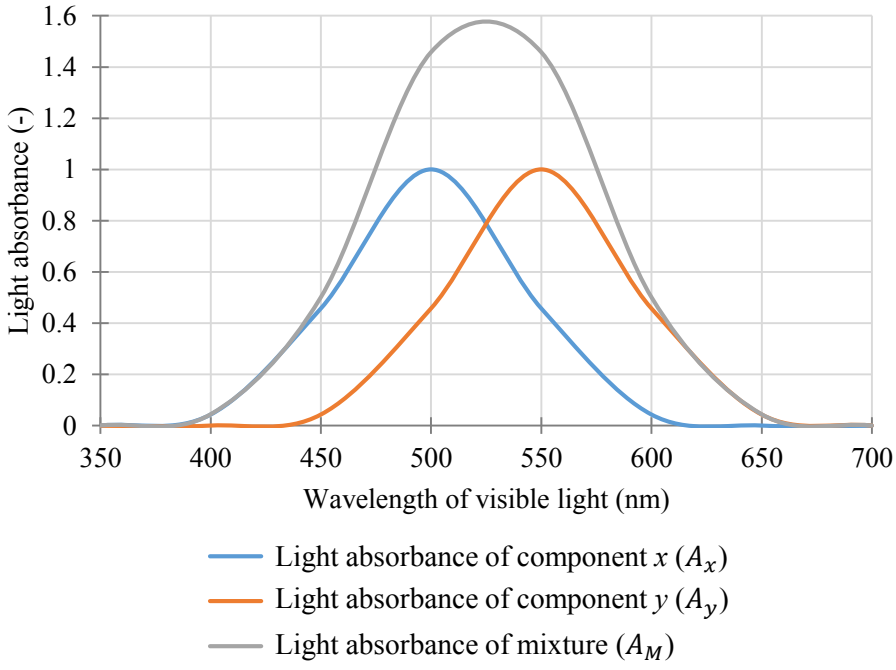


Figure 2.12. Example light absorbance.

The derivative first order of the light absorbance by wavelength is presented in (2.26). In the derivative of light absorbance by wavelength, the concentration of the component and the path length are a constant value. The alteration is only the molar absorptivity.

$$\frac{dA_M}{d\lambda} = c_x l \frac{d\varepsilon_x}{d\lambda} + c_y l \frac{d\varepsilon_y}{d\lambda} \tag{2.26}$$

Figure 2.13 displays the derivative first order of the light absorbance of figure 2.12. It shows that at the wavelength which the light absorbance is the peak of wave, the pure component amplitude of the first order derivative is 0. This point is called "zero-crossing". Therefore, the derivative spectrophotometer method provides the zero-crossing point to eliminate the disinterest component variable. The amplitude of the first-order at the zero-crossing which the light absorbance of the substance *y* is maximum is obtained in (2.27).

$$\frac{dA_M}{d\lambda} = c_x l \frac{d\varepsilon_x}{d\lambda} \tag{2.27}$$

The amplitude of the first order in (2.27) shows that the variable of the component *y* is obliterated and the concentration of the component *x* (c_x) is direct variation with the mixture amplitude of the first order ($\frac{dA_M}{d\lambda}$). Figure 2.14 illustrates the relationship of the first derivative of the mixture light absorbance when the concentration of component *y* increases. It shows that the amplitude of the first derivative at the zero-crossing when the light absorbance of component *y* is at the peak of the wave in zero-order is equal in every solution even if the concentration of the component *y* is changed. Furthermore, figure 2.15 illustrates the amplitude of the first derivative at the zero-crossing when the light absorbance of component *x* is at the peak of the curve in zero order. The amplitude of the first derivative is direct variation with the concentration of the component *y*.

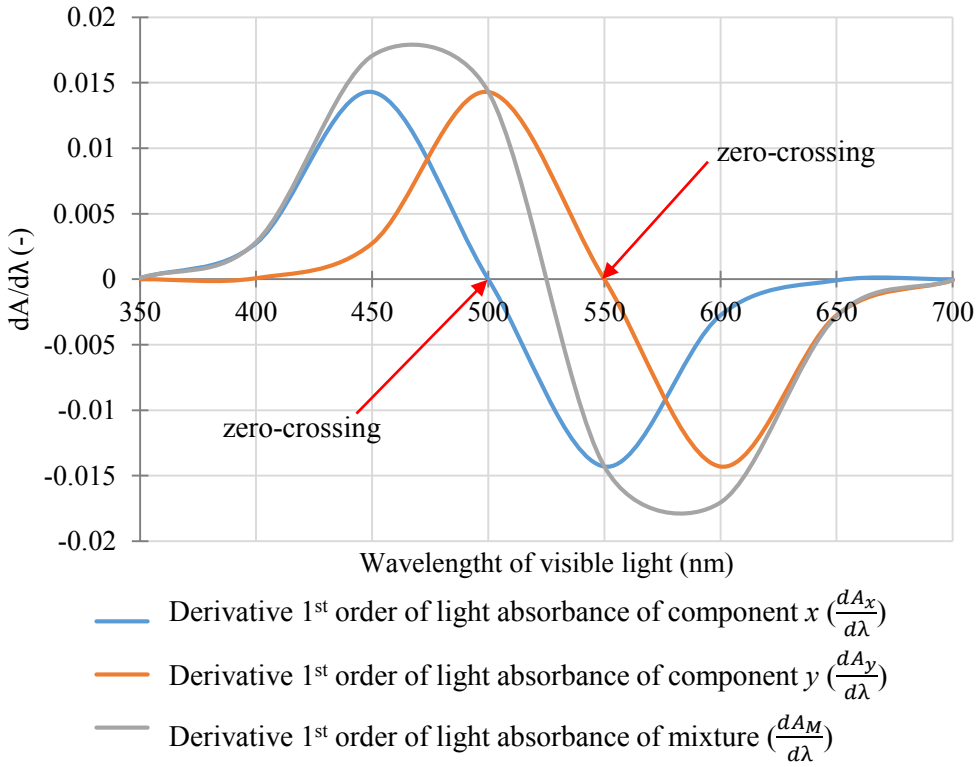


Figure 2.13. First order derivative of example light absorbance.

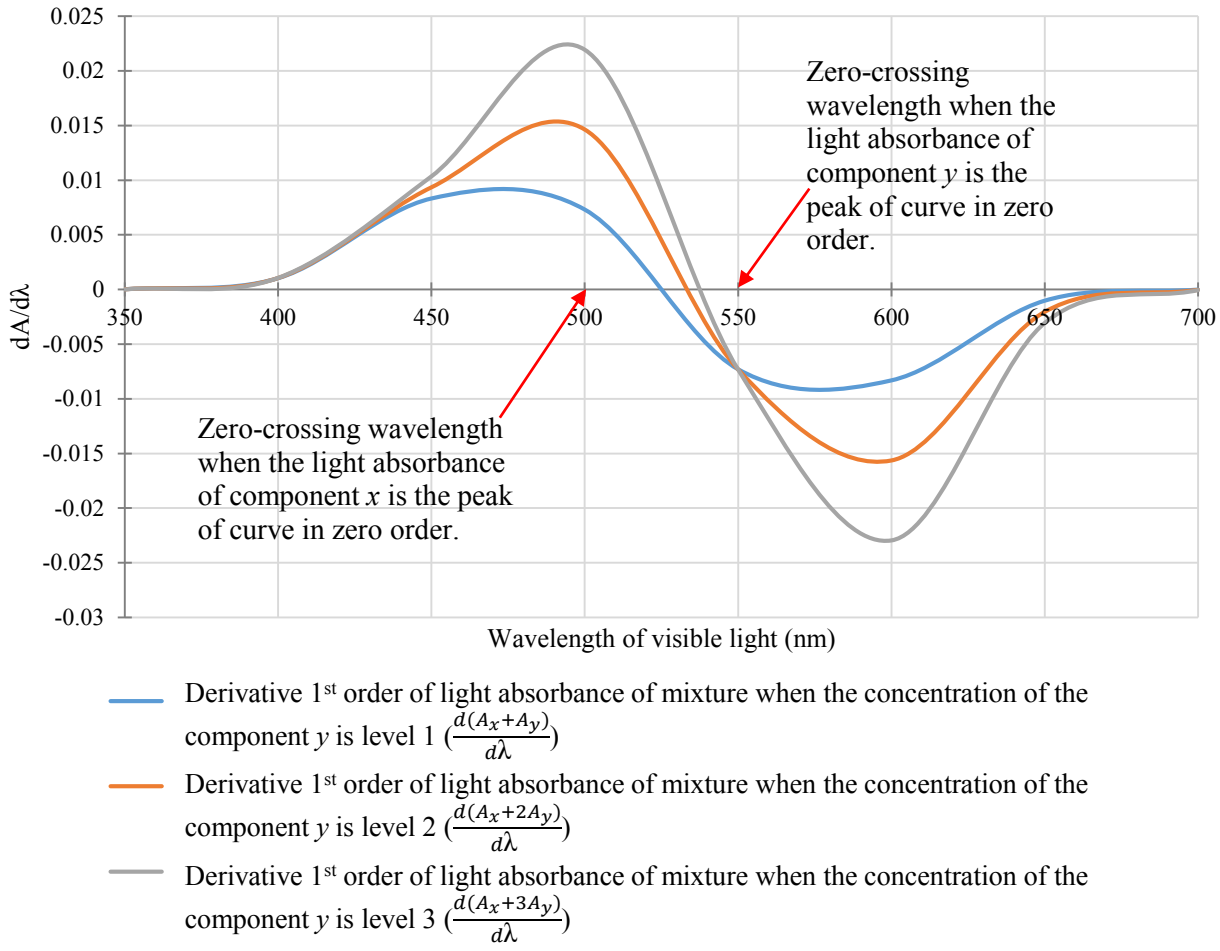


Figure 2.14. First order derivative light absorbance of the mixture solution when the concentration of component *y* increase.

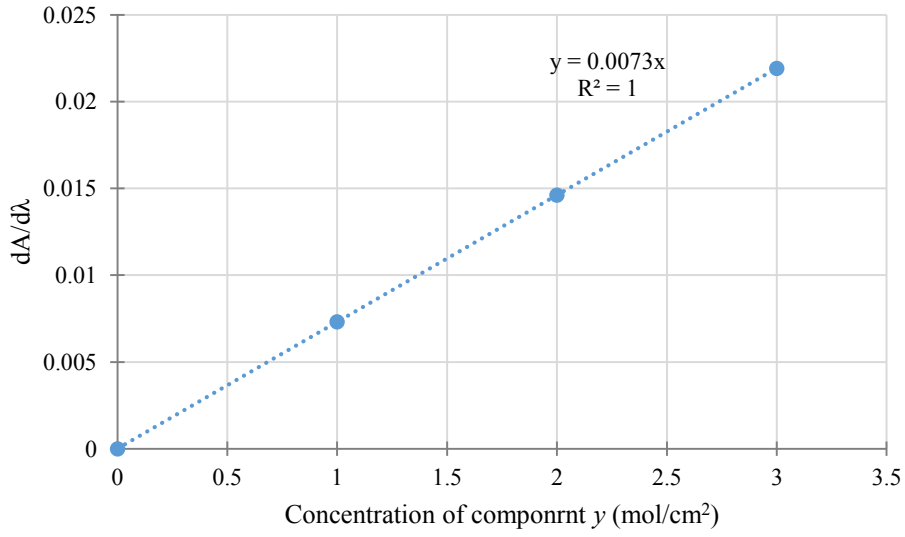


Figure 2.15. Amplitude of the first derivative at the zero-crossing when the light absorbance of component *x* is peak of the curve in zero order.

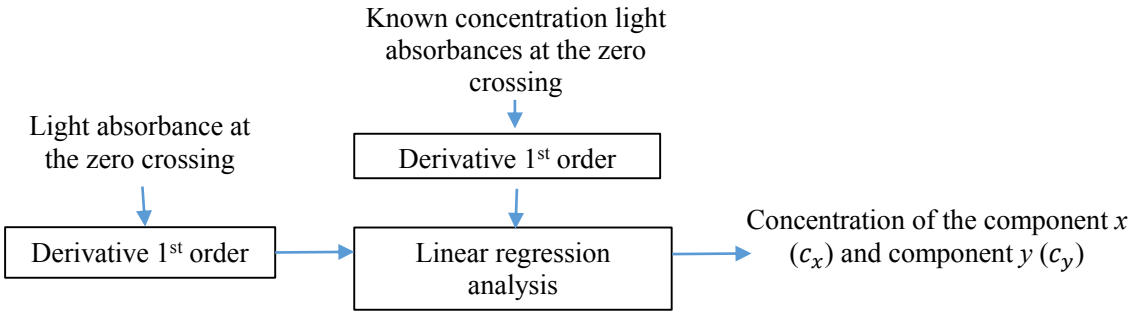


Figure 2.16. Process of the derivative method.

Process of the derivative spectrophotometry is displayed in figure 2.16. The light absorbance at the zero crossing is taken by derivative to eliminate the noise of the disinterest component. Therefore, the derivative first order amplitude of the light absorbance of the mixture is direct variation with the concentration of the interest component at the zero-crossing. The linear regression analysis calculates the linear function that is the relation between the first order amplitude and the concentration of the known concentration solution to calculate the concentration of solution.

2.2.3. Absorb ratio method

This method utilizes the division by the standard solution of the disinterest solution [29-31]. To eliminate the variable of the disinterest solution, the light absorbance ratio between the mixture and the disinterest standard solution is subtracted by the light absorbance ratio between the mixture and the disinterest standard solution of another wavelength. The light absorbance of the mixture solution (A_M) between component *x* and component *y* is the sum of the light absorbance of component *x* (A_x) and *y* (A_y) in (2.28).

$$A_{\mathrm{M}} = A_x + A_y \tag{2.28}$$

The ratio between light absorbance of the mixture solution between component *x* and component *y* (A_M) and the standard solution (A_x^0) of *x* substance is presented in (2.29).

$$\frac{A_M}{A_x^0} = \frac{A_x}{A_x^0} + \frac{A_y}{A_x^0} \tag{2.29}$$

Figure 2.17 shows examples of the light absorbances of component *x*, component *y* and the mixture between component *x* and component *y*. The light absorbance ratio between the standard of the component *x* and mixture solutions is shown in figure 2.18. It shows that the light absorbance ratio between the disinterest component and the standard solution ($\frac{A_x}{A_x^0}$) is constant in every wavelength. Therefore, the difference between the mixture ratio ($\frac{A_M}{A_x^0}$) and the component *y* ratio ($\frac{A_y}{A_x^0}$) is equal in every wavelength. The equation (2.30) is the light absorbance ratio between the mixture and the standard solution of the wavelength 1 is subtracted by the light absorbance ratio between the mixture and the standard solution in wavelength 2.

$$\left[\frac{A_M}{A_x^0}\right]_1 - \left[\frac{A_M}{A_x^0}\right]_2 = \left[\frac{A_y}{A_x^0}\right]_1 - \left[\frac{A_y}{A_x^0}\right]_2 \tag{2.30}$$

When the concentration of the component *y* is factorized from the light absorbance, the equation (2.31) is obtained.

$$\left\{\left[\frac{\varepsilon_y}{A_x^0}\right]_1 - \left[\frac{\varepsilon_y}{A_x^0}\right]_2\right\} c_y = \left[\frac{A_M}{A_x^0}\right]_1 - \left[\frac{A_M}{A_x^0}\right]_2 \quad (2.31)$$

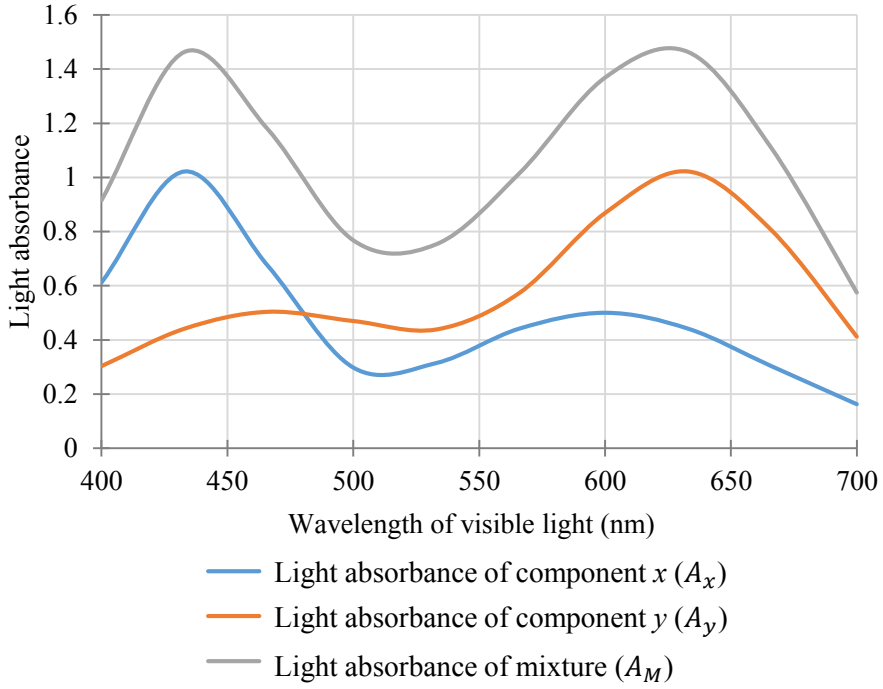


Figure 2.17. Example light absorbance for the absorb ratio method.

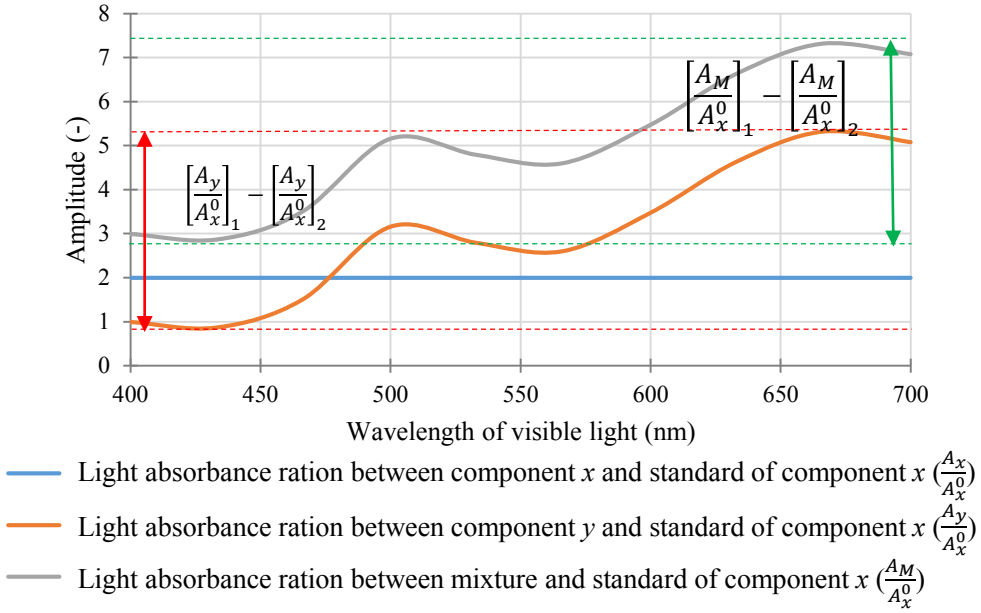


Figure 2.18. Ratio between the light absorbance of component x, component y and mixture and standard of component x.

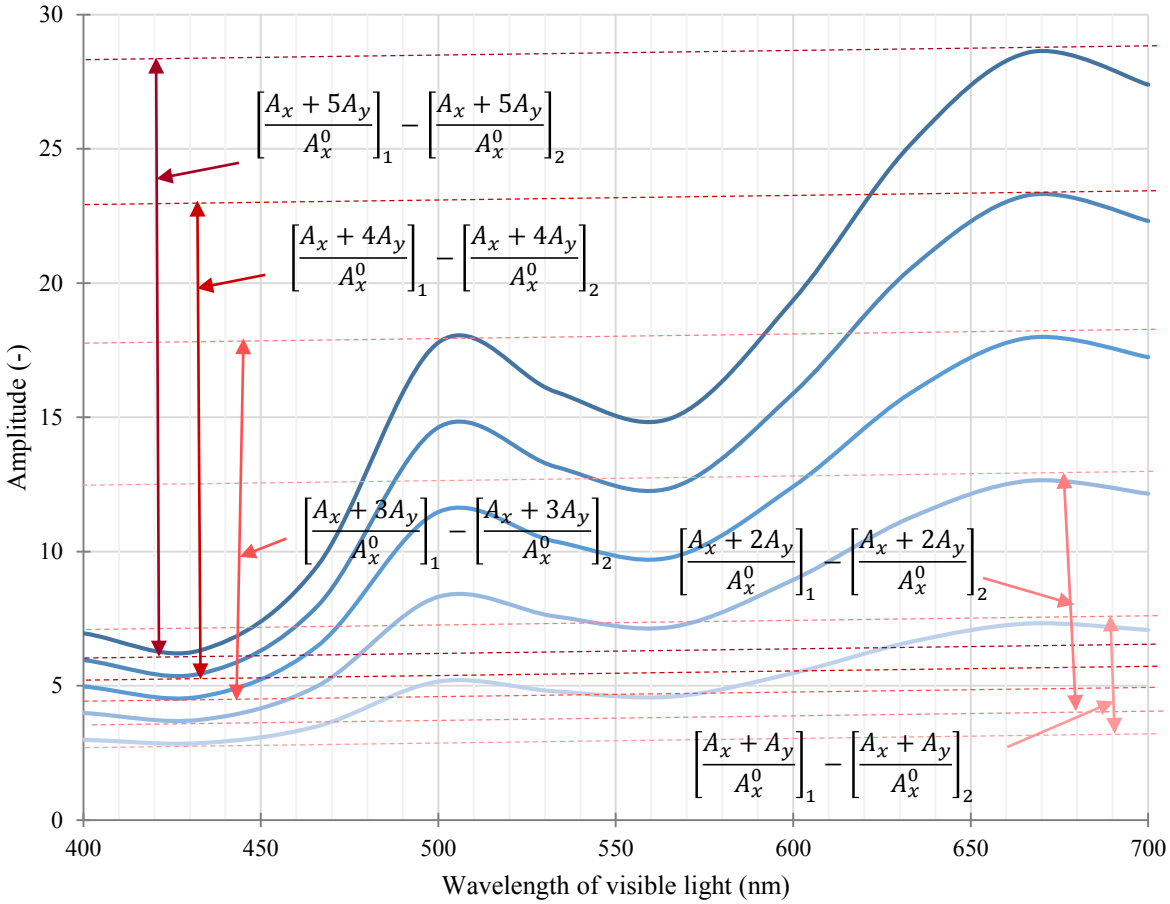


Figure 2.19. Light absorbance ratio between the mixture solution and standard solution of component x when concentration of the component y increases.

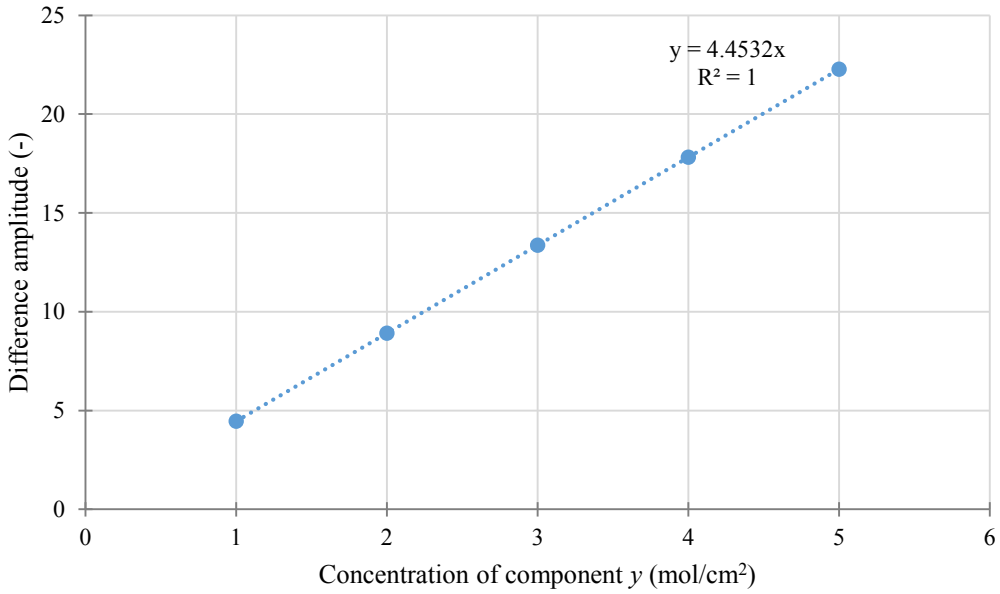


Figure 2.20. Difference of the light absorbance ratio ($\frac{A_x+A_y}{A_x^0}$) between wavelength 1 and wavelength 2 when the concentration of component *y* increases.

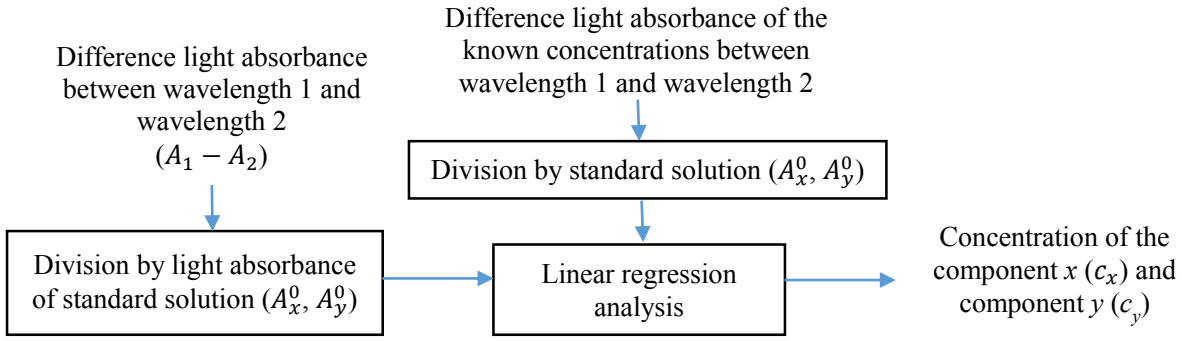


Figure 2.21. Process of the absorbance ratio method.

$\left\{\left[\frac{\varepsilon_y}{A_x^0}\right]_1 - \left[\frac{\varepsilon_y}{A_x^0}\right]_2\right\}$ is constant. Therefore, the concentration of component *y* (c_y) is proportional to the different of the light absorbance ratio between mixture solution and standard of the disinterest component of both wavelengths ($\left[\frac{A_M}{A_x^0}\right]_1 - \left[\frac{A_M}{A_x^0}\right]_2$). Figure 2.19 shows the light absorbance ratio between the mixture solution and the disinterest standard solution of both wavelengths. It demonstrates that when the concentration of the component *y* increases, the difference of the light absorbance ratio ($\frac{A_M}{A_x^0}$) between wavelength 1 and wavelength 2 increases also. The difference of the light absorbance ratio ($\frac{A_M}{A_x^0}$) between wavelength 1 and wavelength 2 ($\left[\frac{A_M}{A_x^0}\right]_1 - \left[\frac{A_M}{A_x^0}\right]_2$) varies with the concentration of the component *y* (c_y) directly shown on figure 2.20. The concentration of the component *y* can be calculated by linear regression analysis.

Process of the absorbance ratio method is exhibited in figure 2.21. The difference of the light absorbance between 2 wavelengths is divided by the disinterest standard solution to eliminate the disinterest component.

Therefore, there is no noise of the disinterest component in the difference of the light absorbance ratio. The difference of the light absorbance ratio varies the concentration of interest component directly. Thus, the linear regression analysis calculates the linear function that is the relation between the difference of the light absorbance ratio and the concentration of the known concentration solution to calculate the concentration of solution.

2.2.4. Derivative ratio spectra method

The derivative ratio spectra method is based on the derivative spectra and the absorbance ratio method [32-36]. This technique continues from (2.29). The derivative first-order of (2.29) by wavelength is obtained in (2.32).

$$\frac{d}{d\lambda}\left[\frac{A_M}{A_x^0}\right] = \frac{d}{d\lambda}\left[\frac{A_x}{A_x^0} + \frac{A_y}{A_x^0}\right] \tag{2.32}$$

The light absorbance ratio between component *x* and the standard of component *x* ($\frac{A_x}{A_x^0}$) is constant. Therefore, it is eliminated. The derivative of the light absorbance ratio is shown in (2.33).

$$\frac{d}{d\lambda}\left[\frac{A_M}{A_x^0}\right] = \frac{d}{d\lambda}\left[\frac{A_y}{A_x^0}\right] \tag{2.33}$$

Figure 2.22 shows the derivative of the light absorbance ratio from figure 2.18. It presents that the derivative of the light absorbance ratio between mixture solution and the standard of the component *x* ($\frac{d}{d\lambda}\left[\frac{A_M}{A_x^0}\right]$) is equal to the light absorbance ratio between component *y* and the standard of the component *x* ($\frac{d}{d\lambda}\left[\frac{A_y}{A_x^0}\right]$). The concentration ($c_y$) and the path length ($l$) are factors of the light absorbance which do not alter by the wavelength in (2.34). Therefore, they can be factorized from the light absorbance, the equation (2.35) is obtained.

$$A_y = c_y l \varepsilon_y \tag{2.34}$$

$$\frac{d}{d\lambda}\left[\frac{A_M}{A_x^0}\right] = c_y l \frac{d}{d\lambda}\left[\frac{\varepsilon_y}{A_x^0}\right] \tag{2.35}$$

It shows that the concentration of the component *y* (c_y) is direct variation with the derivative of the light absorbance ratio between mixture solution and the standard of the component *x* ($\frac{d}{d\lambda}\left[\frac{A_M}{A_x^0}\right]$) in every wavelength. The concentration of component *y* can be calculated by linear regression analysis directly.

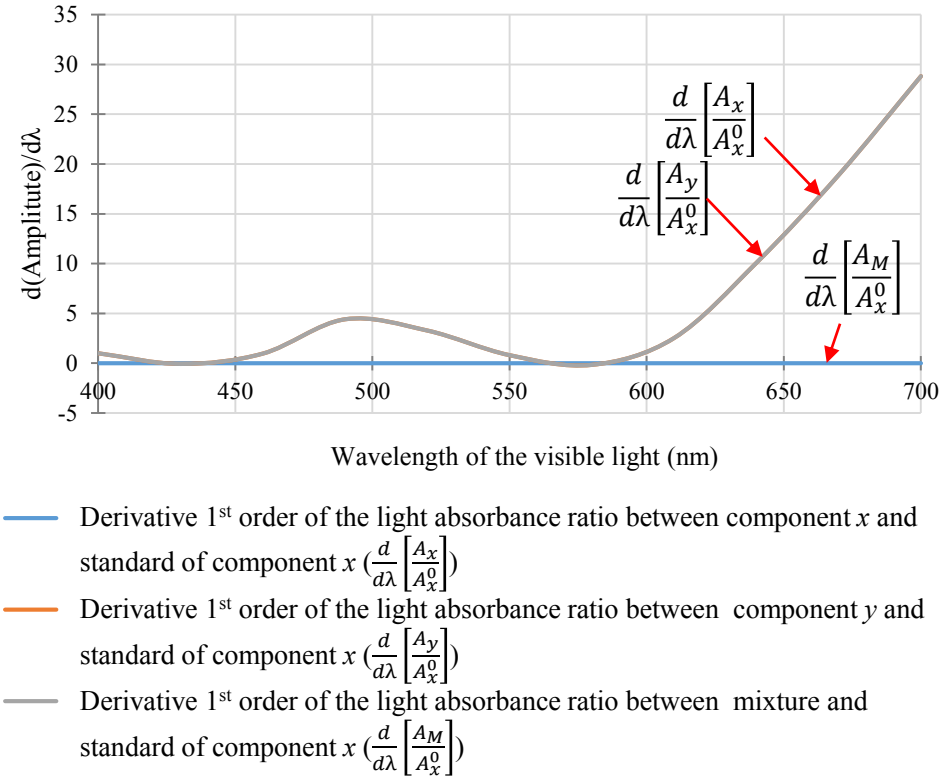


Figure 2.22. Derivative of the ratio of the light absorbance from Figure 2.18.

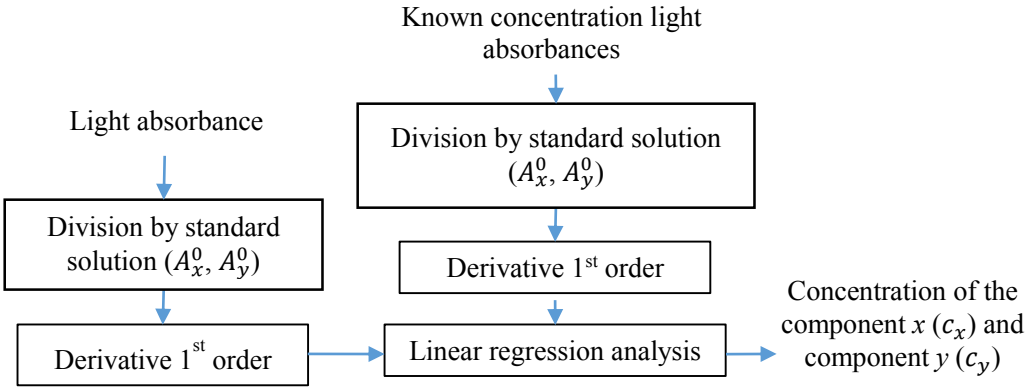


Figure 2.23. Process of the derivative ratio method.

Process of the derivative ratio method is exhibited in figure 2.23. The light absorbance is divided by the disinterest standard solution. After that, to eliminate the disinterest component, the light absorbance ratio is differentiated. The derivative light absorbance ratio does not have the noise of the disinterest component. Therefore, it varies the concentration of solution directly. Thus, the linear regression analysis calculates the linear function that is the relation between the derivative light absorbance ratio and the concentration of the known concentration solution to calculate the concentration of solution.

2.2.5. Double divisor ratio spectra derivative method

This method is based on the derivative ratio spectra method [49-50]. It analyses the 3 compounds of the mixture solution. The light absorbance of 3 compounds mixture solution is shown in (2.36). The mixture consists of component *x*, component *y,* and component *z*.

$$A_M = a_x c_x + a_y c_y + a_z c_z \tag{2.36}$$

It is the same as the absorbance ratio method that provides the standard of disinterest solution. In this method, there are 3 compounds in the mixture. Therefore, the standard solution is the mixture solution of 2 disinterest compounds shown in (2.37).

$$A_M^0 = a_x c_x^0 + a_y c_y^0 \tag{2.37}$$

The ratio between the mixture solution of 3 compounds and the standard mixture of 2 of 3 compounds of the mixture solution ($\frac{A_M}{A_M^0}$) is obtained in (2.38).

$$\frac{A_M}{A_M^0} = \frac{a_x c_x + a_y c_y}{a_x c_x^0 + a_y c_y^0} + \frac{a_z c_z}{a_x c_x^0 + a_y c_y^0} \tag{2.38}$$

The first derivative of the mixture solution of 3 compounds and the standard mixture of 2 of 3 compounds of the mixture solution ($\frac{A_M}{A_M^0}$) is shown in (2.39).

$$\frac{d}{d\lambda}\left[\frac{A_M}{A_M^0}\right] = \frac{d}{d\lambda}\left[\frac{a_x c_x + a_y c_y}{a_x c_x^0 + a_y c_y^0}\right] + \frac{d}{d\lambda}\left[\frac{a_z c_z}{a_x c_x^0 + a_y c_y^0}\right] \tag{2.39}$$

The light absorbance ratio between the 2 compounds which is the mixture and the standard mixture ($\frac{a_x c_x + a_y c_y}{a_x c_x^0 + a_y c_y^0}$) is as constant as to the ratio of disinterest component ($\frac{A_x}{A_x^0}$) in (2.32) in the case of the concentration ratio ($\frac{c_x}{c_y}$) between the component *x* and component *y* is equal with the concentration ration of the standard mixture ($\frac{c_x^0}{c_y^0}$) between component *x* and component *y*. Therefore, the equation (2.40) is obtained.

$$\frac{d}{d\lambda}\left[\frac{A_M}{A_M^0}\right] = \frac{d}{d\lambda}\left[\frac{a_z c_z}{a_x c_x^0 + a_y c_y^0}\right] \tag{2.40}$$

It presents that the concentration of the interest component (c_z) is direct variation with the derivative first order of the light absorbance ratio between the mixture solution (A_M) and the standard mixture solution (A_M^0). Thus, the concentration of the component *z* can be calculated by linear regression analysis with the derivative of the light absorbance ratio ($\frac{d}{d\lambda}\left[\frac{A_M}{A_M^0}\right]$) between the mixture solution and the standard of mixture solution. However, in the case that the ratio of the disinterest component is not equal to the ratio of the disinterest standard component, the equation (2.41) is obtained.

$$\frac{d}{d\lambda}\left[\frac{A_M}{A_M^0}\right] - \frac{d}{d\lambda}\left[\frac{a_x c_x + a_y c_y}{a_x c_x^0 + a_y c_y^0}\right] = \frac{d}{d\lambda}\left[\frac{a_z c_z}{a_x c_x^0 + a_y c_y^0}\right] \tag{2.41}$$

Figure 2.24 displays the light absorbance of the 3 components and the mixture solution of 3 components. Figure 2.25 exhibits the derivative ratio of the light absorbance in figure 2.24. It shows that when the concentration ratio of disinterest component ($\frac{c_x}{c_y}$) is not equal to the concentration ratio of disinterest standard component ($\frac{c_x^0}{c_y^0}$), the derivative of light absorbance ratio between disinterest mixture and disinterest standard

mixture ($\frac{d}{d\lambda}\left[\frac{A_x+2A_y}{A_x^0+A_y^0}\right]$) is not 0. However, when the light absorbance ratio of mixture ($\frac{d}{d\lambda}\left[\frac{A_x+2A_y+A_z}{A_x^0+A_y^0}\right]$) is substituted by the light absorbance ratio of the disinterest solution ($\frac{d}{d\lambda}\left[\frac{A_x+2A_y}{A_x^0+A_y^0}\right]$), the result is equal to the light absorbance ratio of mixture ($\frac{d}{d\lambda}\left[\frac{A_x+A_y+A_z}{A_x^0+A_y^0}\right]$) in case of the ratio of the component is equal to the ratio of the standard component.

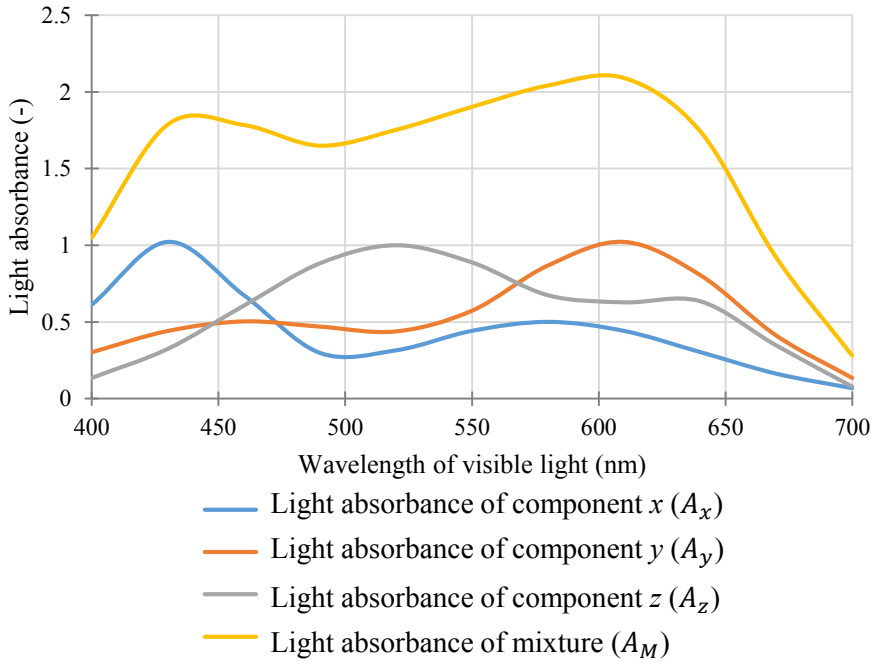
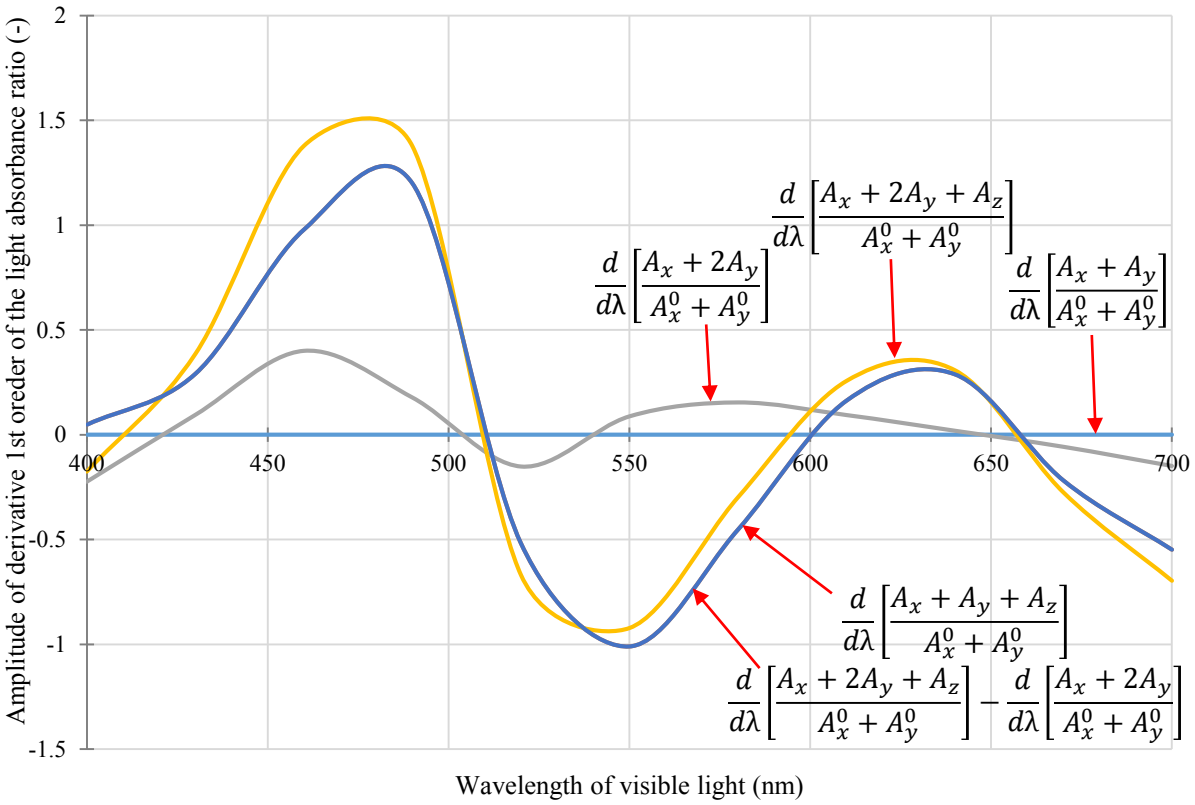


Figure 2.24. Light absorbance of 3 components and the mixture solution of 3 components.



- Derivative 1st order of the light absorbnace ratio between mixture of component *x* and *y* and standard mixture ($\frac{d}{d\lambda}\left[\frac{A_x+A_y}{A_x^0+A_y^0}\right]$)
- Derivative 1st order of the light absorbnace ratio between mixture of component *x*, *y* and z and standard mixture $\frac{d}{d\lambda}\left[\frac{A_x+A_y+A_z}{A_x^0+A_y^0}\right]$)
- Derivative 1st order of the light absorbnace ratio between mixture of component *x* and *y* and standard mixture when the concentration of component *y* is 2 times ($\frac{d}{d\lambda}\left[\frac{A_x+2A_y}{A_x^0+A_y^0}\right]$)
- Derivative 1st order of the light absorbnace ratio between mixture of component *x*, *y* and *z* and standard mixture when the concentration of component *y* is 2 times ($\frac{d}{d\lambda}\left[\frac{A_x+2A_y}{A_x^0+A_y^0}\right]$)
- Derivative 1st order of the light absorbnace ratio between mixture of component *x*, *y* and *z* and standard mixture when the concentration of component *y* is 2 times $\frac{d}{d\lambda}\left[\frac{A_x+2A_y+A_z}{A_x^0+A_y^0}\right]$)

Figure 2.25. Derivative ration light absorbance in the case of 3 components from figure 2.24.

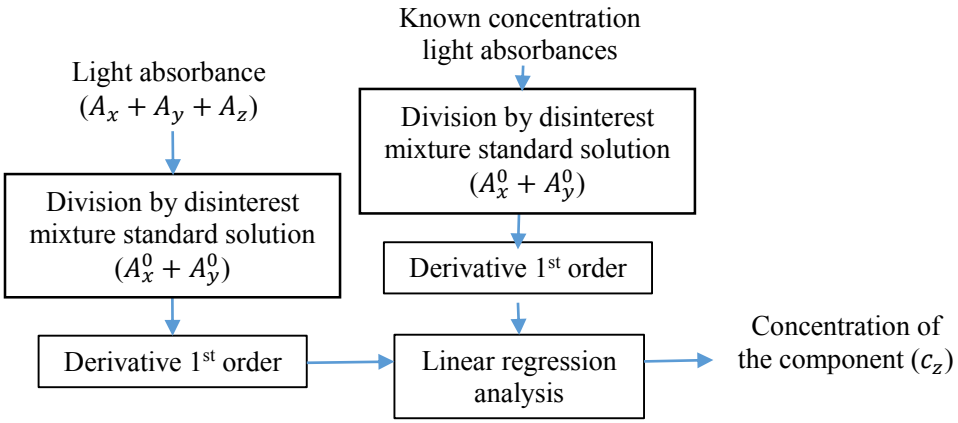


Figure 2.26. Process of the double divisor ratio spectra derivative method.

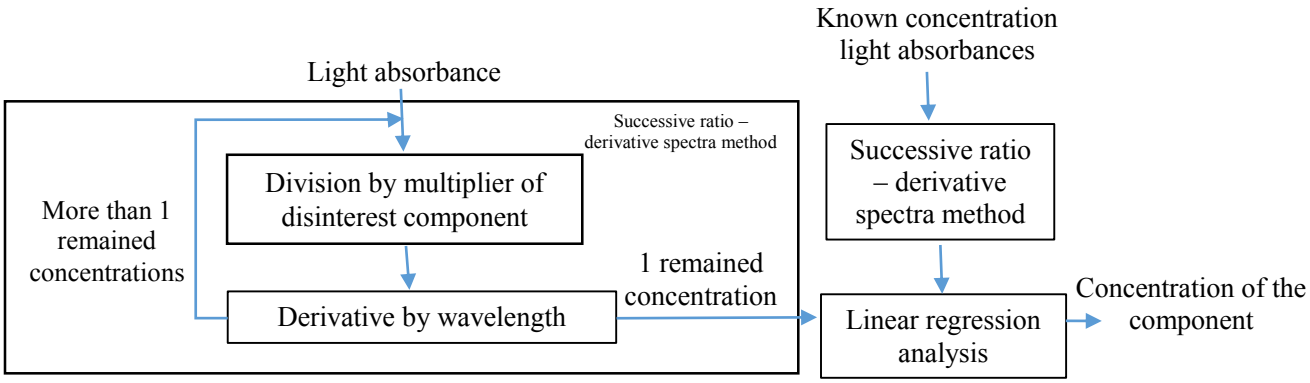


Figure 2.27. Process of the Successive ratio – derivative spectra method.

Process of the double divisor ratio spectra derivative method is the same as the derivative ratio method shown in figure 2.26. The light absorbance is divided by the disinterest mixture standard solution. After that, to eliminate the disinterest mixture component, the light absorbance ratio is differentiated. The derivative light absorbance ratio does not have the noise of the disinterest mixture component. Therefore, it varies the concentration of solution directly. Thus, the linear regression analysis calculates the linear function that is the relation between the derivative light absorbance ratio and the concentration of the known concentration solution to calculate the concentration of solution.

2.2.6. Successive ratio-derivative spectra method

This technique utilizes the derivative to eliminate the disinterest component variables from the light absorbance equation [16]. Therefore, this method can be utilized in the case of more than 3 components in the mixture solution shown in (2.42).

$$A_M = a_x c_x + a_y c_y + a_z c_z \tag{2.42}$$

This method provides the division of the multiplier of the concentration and the derivative by wavelength for eliminating each disinterest component. The concentration is a constant in the alteration of the wavelength. Therefore, it is 0 in the differentiation of wavelength. Before the derivative of the light absorbance

in (2.42), the light absorbance equation in (2.42) is divided by the molar absorptivity of the first disinterest component (a_z) shown in (2.43).

$$\frac{A_M}{a_z} = \frac{a_x c_x + a_y c_y}{a_z} + c_z \tag{2.43}$$

The concentration of component is not changed by the alteration of wavelength. Therefore, the derivative of the first disinterest is eliminated by the wavelength derivative. The derivative equation is obtained in (2.44).

$$\frac{d}{d\lambda}\left[\frac{A_M}{a_z}\right] = \frac{d}{d\lambda}\left[\frac{a_x c_x}{a_z}\right] + \frac{d}{d\lambda}\left[\frac{a_y c_y}{a_z}\right] \tag{2.44}$$

In equation (2.44), there are 2 variables of the component. Next step deletes the second disinterest component. The concentration of the second disinterest component (c_y) is factorized from the light absorbance ($a_y c_y$). After that, the equation (2.44) is divided by the derivative of division $\frac{d}{d\lambda}\left[\frac{a_y}{a_z}\right]$ between the molar absorptivity of the second disinterest component and the molar absorptivity of the first disinterest component shown in (2.45).

$$\frac{\left(\frac{d}{d\lambda}\left[\frac{A_M}{a_z}\right]\right)}{\frac{d}{d\lambda}\left[\frac{a_y}{a_z}\right]} = \frac{\left(\frac{d}{d\lambda}\left[\frac{a_x c_x}{a_z}\right]\right)}{\frac{d}{d\lambda}\left[\frac{a_y}{a_z}\right]} + c_y \tag{2.45}$$

When the first derivative is taken in (2.45), the derivative of the second interest component (c_y) is 0 shown in (2.46) because the concentration does not change in the alteration of the wavelength.

$$\frac{d}{d\lambda}\left[\frac{\left(\frac{d}{d\lambda}\left[\frac{A_M}{a_z}\right]\right)}{\frac{d}{d\lambda}\left[\frac{a_y}{a_z}\right]}\right] = \frac{d}{d\lambda}\left[\frac{\left(\frac{d}{d\lambda}\left[\frac{a_x c_x}{a_z}\right]\right)}{\frac{d}{d\lambda}\left[\frac{a_y}{a_z}\right]}\right] \tag{2.46}$$

As the equation (2.46), there is the linear relationship between the concentration of the interest component (c_x) and $\frac{d}{d\lambda}\left[\frac{\left(\frac{d}{d\lambda}\left[\frac{A_M}{a_z}\right]\right)}{\frac{d}{d\lambda}\left[\frac{a_y}{a_z}\right]}\right]$. The linear regression analysis calculates the linear function that is the relationship between the concentration of the interest component (c_x) and $\frac{d}{d\lambda}\left[\frac{\left(\frac{d}{d\lambda}\left[\frac{A_M}{a_z}\right]\right)}{\frac{d}{d\lambda}\left[\frac{a_y}{a_z}\right]}\right]$ by known concentration data to calculate the concentration of solution of interest component (c_x). Since the sensitivity of $\frac{d}{d\lambda}\left[\frac{\left(\frac{d}{d\lambda}\left[\frac{A_M}{a_z}\right]\right)}{\frac{d}{d\lambda}\left[\frac{a_y}{a_z}\right]}\right]$, the maximum or minimum wavelength should be measured.

The process of the successive ratio – derivative spectra method is exhibited in figure 2.27. This process eliminates the variable of the component until the remained variable is 1. The elimination is the division of the multiplier of the disinterest concentration and the derivative by wavelength. When the variable of the component remains 1, the output varies the concentration of the remained component directly. Therefore, the linear regression analysis calculates the linear function that is the relation between the output and the concentration of the known concentration solution to calculate the concentration of solution.

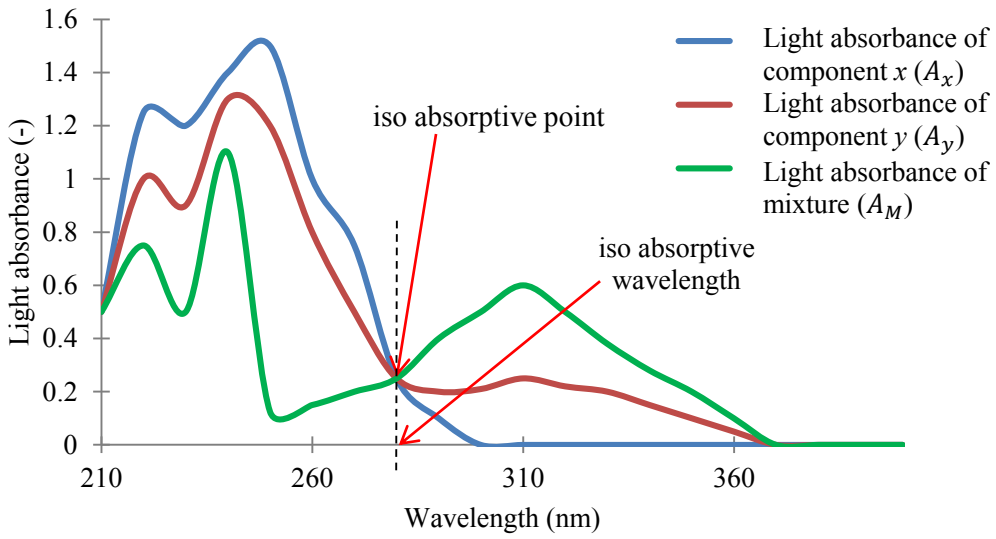


Figure 2.28. Light absorbance in many spectra of *x, y* and mixture solution between *x* and *y* the concentration of *x* and *y* is half of one concentration in ratio 1:1.

2.2.7. Isosbestic "isoabsorptive" point method

This method requires the specific case of the concentration. It is the isosbestic or iso-absorptivity point. At this point, the molar absorptivity of each component in the mixture is equal [37-40]. Figure 2.28 displays the iso-absorptive point of the solution *x* and *y* in the wavelength between 210-400 nm. At the iso-absorptive point, the light absorbances of the solution *x* and solution *y* are equal.

Furthermore, the mixture of both components which the concentration of each component is half of the pure solution is equal to the concentration of the pure solution. It shows that the molar absorptivity of solution *x* and solution *y* are equal. Therefore, the light absorbance can be calculated by (2.47).

$$A_M = \varepsilon_M l(c_x + c_y) = \varepsilon_M c_M l \tag{2.47}$$

The concentration of the mixture solution (c_M) is the sum of the concentration of all components shown in (2.48). When the concentration of one component is known, the concentration of another component is known also. Therefore, it provides the other method to calculate the one concentration only.

$$c_M = c_x + c_y \tag{2.48}$$

2.2.8. Absorptivity factor method

This method is modified from the iso-absorptive method. However, in this case, it does not provide the iso-absorptive point. This method provides the absorptivity factor point [47-48]. This point looks like the iso-absorptive point which the light absorbances of both devices are equal. However, the molar absorptivity is not necessary to be equal. The absorptivity factor point in the wavelength between 200-400 nm is illustrated in figure 2.29. When the light absorbances of both solutions are equal shown in (2.49). The light absorbance of the mixture solution can be calculated by (2.50).

$$A_x = A_y \tag{2.49}$$

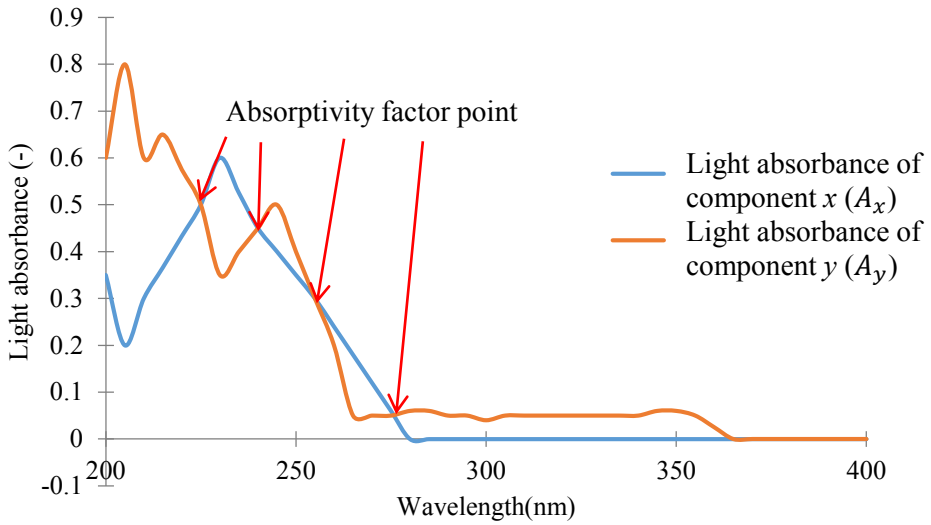


Figure 2.29. Absorptivity factor point.

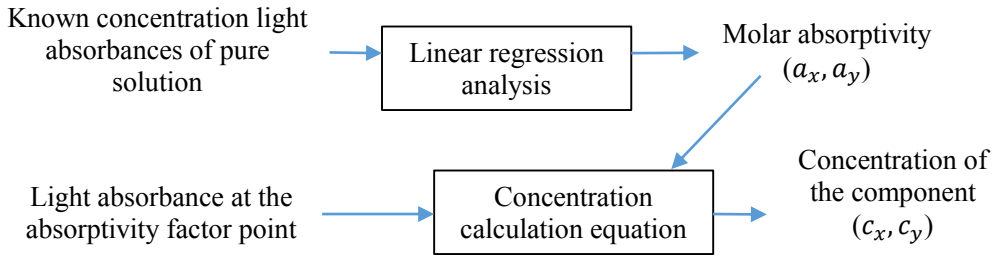


Figure 2.30. Process of the absorptivity factor method.

$$A_M = A_x + A_y = 2A_x = 2A_y \tag{2.50}$$

When the light absorbance of the mixture is rewritten into the Beer-Lambert's law, the equation (2.51) and (2.52) are obtained.

$$A_M = 2c_x a_x \tag{2.51}$$

$$A_M = 2c_y a_y \tag{2.52}$$

Therefore, the concentration can be calculated by (2.53) and (2.54).

$$c_x = \frac{A_M}{2a_x} \tag{2.53}$$

$$c_y = \frac{A_M}{2a_y} \tag{2.54}$$

The process of the absorptivity factor method exhibited in figure 2.30 is the same as the concentration calculation of pure solution. Therefore, it requires the molar absorptivity from the linear regression analysis which is calculated by the light absorbance and the concentration of the known concentration pure solution.

2.2.9. Q-absorbance ratio method

This method is also termed of the absorbance ratio method. It is modified from the simultaneous equation method. This method requires the ratio of light absorbance between both methods shown in (2.55) [41-46]. The two wavelengths are the wavelength 2 which are the highest light absorbance of one of the components and the wavelength 1 of the iso-absorptive point which the light absorbances of two components are equal shown in figure 2.28.

$$\frac{A_{M_2}}{\mathrm{A_{M_1}}} = \frac{A_{x_2}+A_{y_2}}{A_{x_1}+A_{y_1}} \tag{2.55}$$

When the light absorbance is represented by Beer's law's which the path length is 1 cm, the rewritten equation is shown in (2.56).

$$\frac{A_{M_2}}{\mathrm{A_{M_1}}} = \frac{a_{x_2}c_x+a_{y_2}c_y}{a_{x_1}c_x+a_{y_1}c_y} \tag{2.56}$$

At the iso-absorptive point, the molar absorptivity of both solutions is equal. Therefore, a_{x_1} is equal to a_{y_1}. The equation becomes (2.57).

$$\frac{A_{M_2}}{\mathrm{A_{M_1}}} = \frac{a_{x_2}c_x}{a_{x_1}(c_x+c_y)} + \frac{a_{y_2}c_y}{a_{y_1}(c_x+c_y)} \tag{2.57}$$

To make the easily observable equation, F_x and F_y from (2.58) and (2.59) are substituted into (2.57), respectively. The equation (2.60) is obtained.

$$F_x = \frac{c_x}{c_x+c_y} \tag{2.58}$$

$$F_y = \frac{c_y}{c_x+c_y} \tag{2.59}$$

$$\frac{A_{M_2}}{A_{M_1}} = \frac{a_{x_2}F_x}{a_{x_1}} + \frac{a_{y_2}F_y}{a_{y_1}} \tag{2.60}$$

The concentration of solution is a factor of the light absorbance which is not altered by the wavelength. Therefore, when the equation (2.61) is taken by 1 ($\frac{c_x}{c_x}, \frac{c_y}{c_y}$), the equation (2.62) is obtained.

$$\frac{A_{M_2}}{\mathrm{A_{M_1}}} = \frac{c_x a_{x_2}F_x}{c_x a_{x_1}} + \frac{c_y a_{y_2}F_y}{c_y a_{y_1}} \tag{2.61}$$

$$\frac{A_{M_2}}{\mathrm{A_{M_1}}} = \frac{A_{x_2}F_x}{A_{x_1}} + \frac{A_{y_2}F_y}{A_{y_1}} \tag{2.62}$$

Each ratio is shown in (2.63), (2.64) and (2.65).

$$Q_M = \frac{A_{M_2}}{\mathrm{A_{M_1}}} = \frac{Absorbance\ of\ the\ sample\ solution\ at\ the\ wavelength\ 2\ of\ one\ of\ the\ components}{Absorbance\ of\ the\ sample\ solution\ at\ wavelength\ 1} \tag{2.63}$$

$$Q_x = \frac{A_{x_{\lambda_2}}}{A_{x_{\lambda_1}}} = \frac{a_{x_{\lambda_2}}}{a_{x_{\lambda_1}}} = \frac{Absorbnace\ or\ molar\ absorptivity\ of\ x\ at\ the\ wavelength\ 2\ \ of\ one\ of\ the\ components}{Absorbnace\ or\ molar\ absorptivity\ \ of\ x\ at\ the\ wavelength\ 1} \tag{2.64}$$

$$Q_y = \frac{A_{y_{\lambda_2}}}{A_{y_{\lambda_1}}} = \frac{a_{y_{\lambda_2}}}{a_{y_{\lambda_1}}} = \frac{Absorbnace\ or\ molar\ absorptivity\ \ of\ y\ at\ the\ wavelength\ 2\ \ of\ one\ of\ the\ components}{Absorbnace\ or\ molar\ absorptivity\ \ of\ y\ at\ the\ wavelength\ 1} \tag{2.65}$$

When the equation (2.63), (2.64) and (2.65) substitute in (2.62), the rearranged equation is shown in (2.66). The sum between F_x and F_y is 1 shown in (2.67).

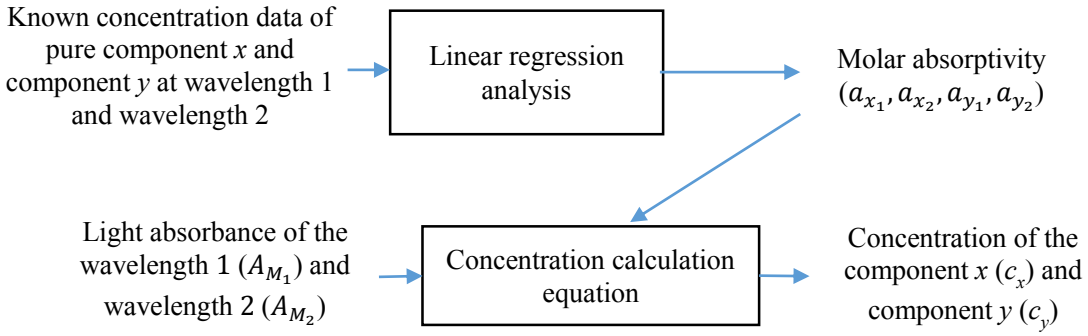


Figure 2.31. Process of the Q-absorbance ratio method.

$$Q_M = Q_x F_x + Q_y F_y \tag{2.66}$$

$$F_x + F_y = 1 \tag{2.67}$$

The equation (2.66) is rewritten by (2.68) and (2.69) that is the concentration of the component x and component y, respectively.

$$c_x = \frac{(Q_M - Q_y)A_{M_1}}{(Q_x - Q_y)a_{x_1}} \tag{2.68}$$

$$c_y = \frac{(Q_M - Q_x)A_{M_1}}{(Q_y - Q_x)a_{y_2}} \tag{2.69}$$

The process of the Q-absorbance ratio method is displayed in figure 2.31. The process is the same as the simultaneous equation method which utilizes the molar absorptivity of all cases and the light absorbance of 2 wavelengths. The calculation equation is shown in (2.68) and (2.69).

2.3. Comparison of the previous spectrophotometric method

This section compares the previous spectrophotometric methods in the case of the 2 components. Therefore, there are no the double divisor ratio spectra derivative method and the successive ratio – derivative spectra method that is utilized with the more 3-component solution. In the ideal case, the results of all concentration are perfect. The criteria comparison is about the number of inputs, linear regression calculation times, and the specific condition. The linear regression analysis is provided to calculate the molar absorptivity or make a linear function that is the relationship between the outputs of each method and the concentration of solution to calculate the concentration of the unknown concentration solution.

Table 2.1 exhibits the number of light absorbance, the number of molar absorptivity, linear regression calculation time, derivative time, and specific condition in the calculation of 1 component from the 2 components solution. Table 2.2 shows the number of light absorbance, the number of molar absorptivity, linear regression calculation time, derivative time, and specific condition in the calculation of the 2 components from the 2 components solution. It shows that the calculation of each component employs the same variable in some methods.

The simultaneous equation requires all molar absorptivity and the light absorbance of 2 wavelengths to calculate the concentration of 2 components. Therefore, the calculation of each component required the same variable. Furthermore, it can be used in every spectrophotometer without specific condition.

The derivative spectrophotometry provides only 1 light absorbance at the zero-crossing to calculate the concentration of one component. The derivative of the light absorbance at the zero-crossing does not have the noise of the one component. Therefore, the concentration can be calculated by the linear regression analysis between the light absorbance at the zero-crossing and the concentration of another components. The molar absorptivity is not essential in the calculation of this method. However, the derivative function is necessary. Thus, the limited wavelength spectrophotometer cannot employ this method.

The absorptivity ratio requires the light absorbance of standard solution of the disinterest component. The noise of the disinterest component is eliminated when the light absorbance is subtracted with the light absorbance of another wavelengths. The concentration of interest component varies the difference of the light absorbance ration between mixture and disinterest concentration of both wavelengths. The standard solution does not have to equal with molar absorptivity. In the error case, if the standard solution is equal with the molar absorptivity, the result is the same as the result of the simultaneous equation.

The derivative ratio spectra method is modified from the absorb ratio and the derivative ratio. It eliminates the noise of the disinterest component by the division of the light absorbance of standard solution and the derivative by wavelength. The derivative of the light absorbance ratio between the mixture and standard disinterest solution is direct variation with the concentration of the interest concentration in every wavelength. Therefore, it does not employ the specific condition and the molar absorptivity. To calculate the concentration of 2 components, one light absorbance is provided only. However, because of the same as the derivative method, the derivative function is necessary.

The isosbestic point method provides the other methods to calculate the concentration of one component. When the concentration of one component is known, the concentrations of another components are known without the other variables.

The absorptivity factor method provides the 1 light absorbance to calculate the concentration of the 2 components. At the absorptivity factor point, the light absorbance of every component is equal. Therefore, to calculate the concentration, it provides only the one molar absorptivity per component.

Q-absorbance ratio method can calculate the concentration of the solution 2 ways. The first way uses 2 light absorbances and 4 molar absorptivitys. The second way uses 6 light absorbances and 1 molar absorptivity. Furthermore, this method provides the isosbestic point.

From the comparison, to calculate the concentration of the component, the necessity is the linear regression analysis. To calculate the molar absorptivity or the calculation of the concentration in the last process, the linear regression analysis is required. The derivative requires many wavelengths of light. The limited wavelength spectrophotometer cannot utilize derivative. Furthermore, the specific condition is only in some wavelengths which can be found by multi-wavelength spectrophotometer. Therefore, there are 2 methods which can be used in every spectrophotometer without specific condition. They are simultaneous equation method and the absorbance ratio.

From the above previous spectrophotometric method, they can be categorized to 2 main methods. The first is the use of all molar absorptivity between the component and the wavelength to calculate the concentration of solution. The second is the elimination of noise of the disinterest concentration which the remained value varies the concentration of the interest concentration directly.

Table 2.1. Comparison of the previous spectrophotometric method in case of the calculation of concentration of one component in 2-component solution.

Method	Number of the light absorbance	Number of the molar absorptivity	Linear regression calculation time	Derivative time	Specific condition
Simultaneous equation method	2	4	4	-	-
Derivative spectrophotometry	1	-	1	1	Zero-crossing
Absorbance ratio method	2	- (2)	1 (3)	-	-
Derivative ratio spectra method	1	1	2	1	-
Isosbestic point method	Dependent the used method	Dependent the used method	Dependent the used method	Dependent the used method	Isosbestic point
Absorptivity factor method	1	1	1	-	Absorptivity factor
Q-absorbance ratio method	2	1 (4)	1 (4)	-	Isosbestic point

Table 2.2. Comparison of the previous spectrophotometric method in case of the calculation of concentration of two components in 2-component solution.

Method	Number of the light absorbance	Number of the molar absorptivity	Linear regression calculation time	Derivative time	Specific condition
Simultaneous equation method	2	4	4	-	-
Derivative spectrophotometry	2	-	2	2	Zero-crossing
Absorbance ratio method	2	- (4)	2 (6)	-	-
Derivative ratio spectra method	1	2	4	2	-
Isosbestic point method	Same as calculation of one component	Same as calculation of one component	Same as calculation of one component	Same as calculation of one component	Isosbestic point
Absorptivity factor method	1	2	2	-	Absorptivity factor
Q-absorbance ratio method	2	2 (4)	2 (4)	-	Isosbestic point

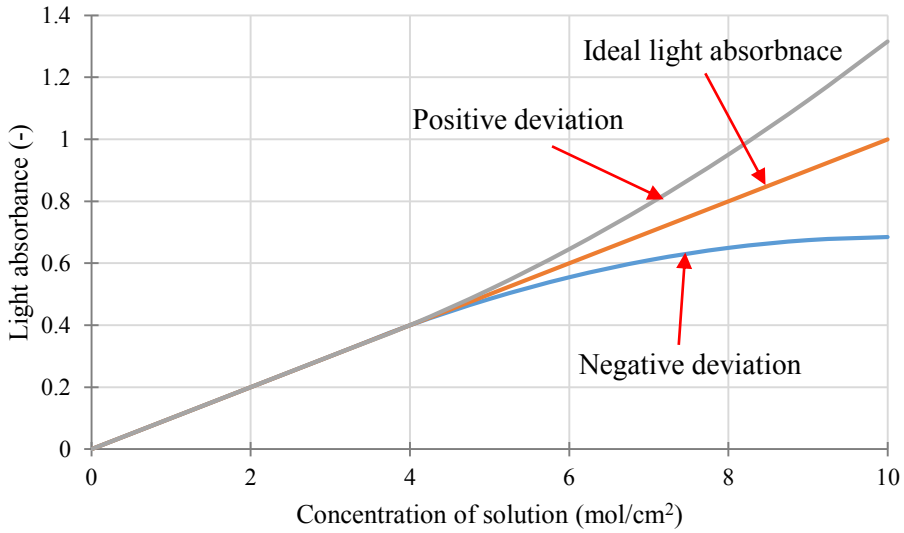


Figure 2.32. Deviations from the Beer's law.

In the concentration calculation only 1 component, the noise elimination method provides less the linear regression calculation time than the method using all molar absorptivity. However, in the concentration calculation 2 components without specific component, the linear regression calculation time of the noise elimination method and the method using all molar absorptivity are equal.

2.4. Deviation of the Beer-Lambert's law

In the ideal case, the calculated concentrations of the components are equal in all methods. However, in the experiment, there are many errors which are not following the Beer-Lambert's law [55,60]. It is called the deviation of Beer-Lambert's law. There are the positive and negative deviations shown in figure 2.32. These deviations from the Beer-Lambert's law can be classified into three categories. There are real deviations, chemical deviations, and instrument deviations.

2.8.1. Real deviation

Beer-Lambert's law describes the relationship between the light absorbance and the concentration of the solution capable at the low concentration which is the concentration of solution is lesser than 0.01 M. When the concentration of solution is more than 0.01M, the deviations are due to interactions between the absorbing species and to alterations of the refraction of the medium.

At the high concentration, the molecules of the solute are a cause to change the distribution on their neighboring species in the solution differently. Because the light absorbance is an electronic phenomenon, the high concentration would possibly result in a shift in the absorption wavelength. In some time, the electrolyte concentration changes the distributions and affecting the light absorbance. Some large ion or molecule present the deviation even at the low concentration. Furthermore, the high concentration can alter the refractive index of the solution which affects the light absorbance.

2.8.2. Chemical deviation

The chemical deviation occurs by the chemical phenomenon involving association, dissociation, and interaction of the molecule with the solvent to produce a product with different absorption characteristics.

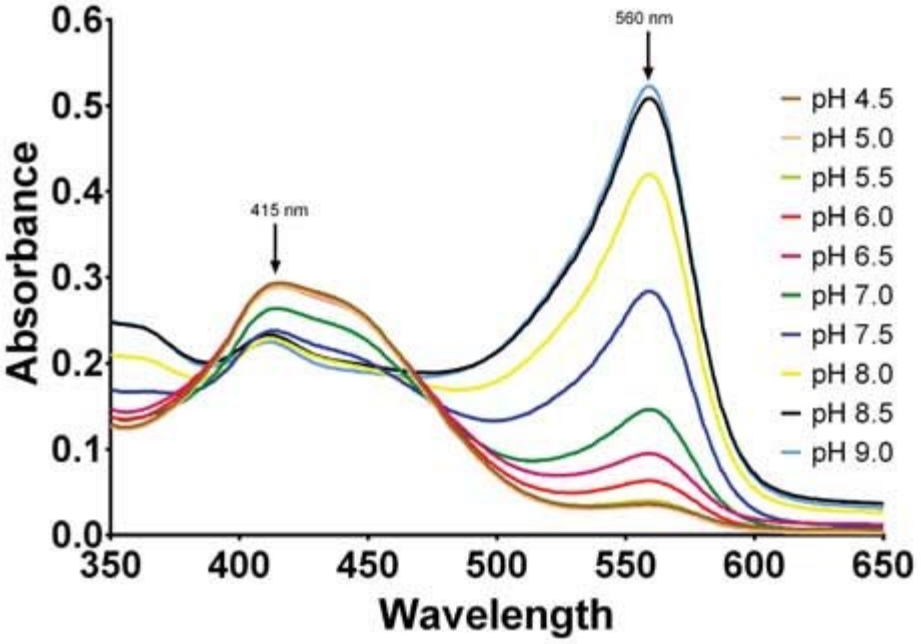


Figure 2.33. Light absorbance of phenol red.
(Picture from Using Phenol Red to Assess pH in Tissue Culture Media [61])

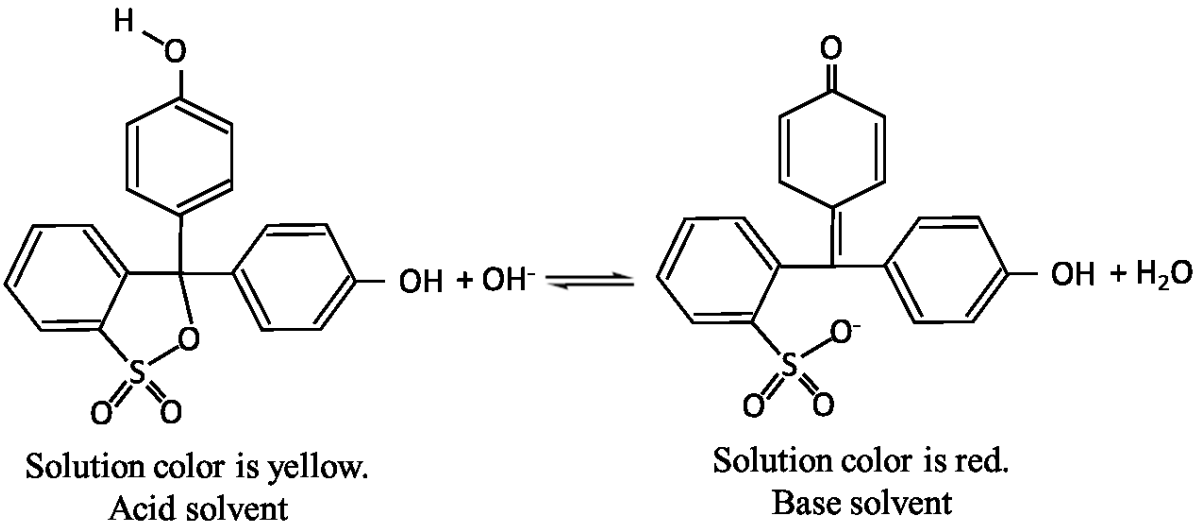


Figure 2.34. Transformation of the molecule of the phenol red.

For an example, figure 2.33 shows the light absorbance of the phenol red in any pH level of the solvent. When pH of the solvent is changed, the light absorbance of the phenol red is changed. When the solvent is acid, the molecule of the phenol red associates with hydrogen ion (H^+) of the solvent. The color of the phenol red is altered from red to yellow. When the solvent is changed from the acid to the base the hydrogen ion (H^+) dissociates from the molecule of the phenol red to associate with the hydroxide (OH^-), the color of the phenol red become red from yellow. Transformation of the molecule of the phenol red is shown in figure 2.34.

2.8.3. Instrumental deviation

The instrumental deviation occurs due to the instrument in the experiment. Beer-lambert's law is strict about a light source which is the monochromatic light. Monochromators are used to isolate portions of the output from continuum light sources. However, the truly monochromatic radiation never exists. It can only be approximated the wavelength of the light which is the polychromatic light. When the incident light consists of the 2 wavelengths, the incident light intensity (I_{0_M}) is calculated by (2.70).

$$I_{0_M} = I_{0_{\lambda_1}} + I_{0_{\lambda_2}} \tag{2.70}$$

As the same with the incident light intensity, the transmitting light (I_M) of 2 wavelengths is the sum of the transmitting light intensity of the 2 wavelengths shown in (2.71).

$$I_M = I_{\lambda_1} + I_{\lambda_2} \tag{2.71}$$

The transmittance (T_M) of the 2 wavelengths is determined by the ratio between the transmitting light intensity and the incident light intensity shown in (2.72).

$$T_M = \frac{I_M}{I_{0_M}} = \frac{I_{\lambda_1}+I_{\lambda_2}}{I_{0_{\lambda_1}}+I_{0_{\lambda_2}}} \tag{2.72}$$

Therefore, the light absorbance by the 2 wavelengths is calculated by the minus logarithm of the transmittance shown in (2.73).

$$A_M = -\log(T_M) = -\log[\frac{I_{\lambda_1}+I_{\lambda_2}}{I_{0_{\lambda_1}}+I_{0_{\lambda_2}}}] \tag{2.73}$$

The light absorbance at the wavelength λ_1 and λ_2 is calculated by (2.74) and (2.75), respectively.

$$A_{\lambda_1} = -\log(\frac{I_{\lambda_1}}{I_{0_{\lambda_1}}}) \tag{2.74}$$

$$A_{\lambda_2} = -\log(\frac{I_{\lambda_2}}{I_{0_{\lambda_2}}}) \tag{2.75}$$

The equation (2.74) and (2.75) is rewritten by (2.76) and (2.77) to calculate the transmitting light.

$$I_{\lambda_1} = I_{0_{\lambda_1}} 10^{-A_{\lambda_1}} \tag{2.76}$$

$$I_{\lambda_2} = I_{0_{\lambda_2}} 10^{-A_{\lambda_2}} \tag{2.77}$$

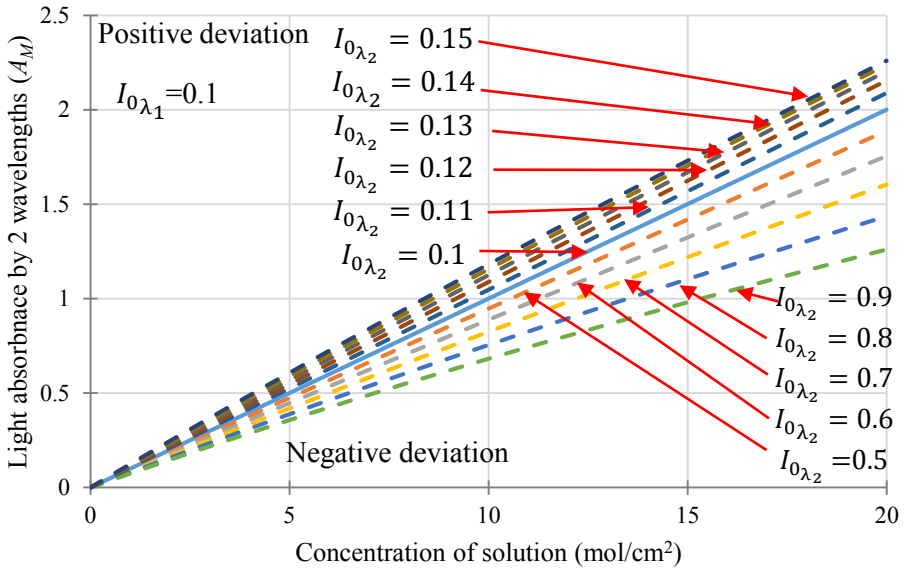


Figure 2.35. Light absorbance by 2 wavelengths when the molar absorptivity of one wavelength is changed.

When the transmitting light intensity of λ_1 and λ_2 (I_{λ_1},I_{λ_2}) is taken into the (2.73), the light absorbance by 2 wavelengths (A_M) is calculated by (2.78).

$$A_M = \log[\frac{I_{0\lambda_1}+I_{0\lambda_2}}{I_{0\lambda_1}10^{-A_{\lambda_1}}+I_{0\lambda_2}10^{-A_{\lambda_2}}}] \tag{2.78}$$

When the molar absorptivity of both wavelengths is the same, the relationship between the absorbance and the concentration of solution follows Beer-Lambert's law to obtain a straight line. However, the difference between the molar absorptivity of both wavelengths ($\varepsilon_{\lambda_1}, \varepsilon_{\lambda_2}$) increases, the deviations from linearity also increases. Figure 2.35 illustrates the light absorbance when the molar absorptivity of one wavelength is changed.

The polychromatic light occurs by the many wavelengths of light going to the solution. In the measurement, a slit covers a light source. Therefore, a slit is narrow. The wavelength of light going to the solution is lesser. However, in the experiment, a slit is constant. Therefore, to reduce the deviation to a minimum, the selection of the wavelength is required. Figure 2.36 shows the 2 bands of the wavelength. The band A is wavelength which is a minimal change of the molar absorptivity (λ_{MAX}). Figure 2.37 shows the deviations from Beer-Lambert's laws which is observed at the maximum wavelength (λ_{MAX}) and the other wavelength. It shows that the relationship between the concentration and light absorbance of the band A which is the maximum wavelength (λ_{MAX}) is linear relation. However, the relationship between the concentration and light absorbance of the band B which has a large change of the molar absorptivity is non-linear relation. This is a reason why the measurement of the light absorbance requires the maximum wavelength.

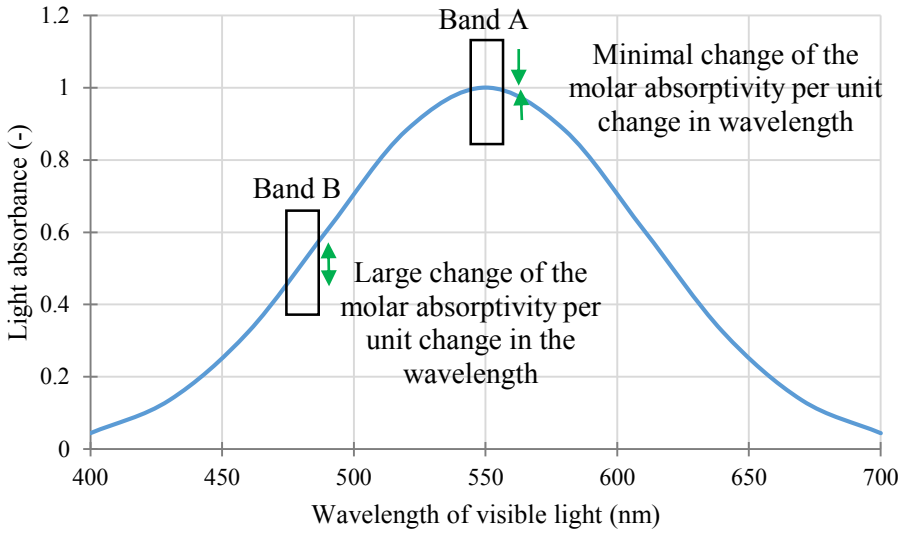


Figure 2.36. Change of the molar absorptivity per unit change in the wavelength.

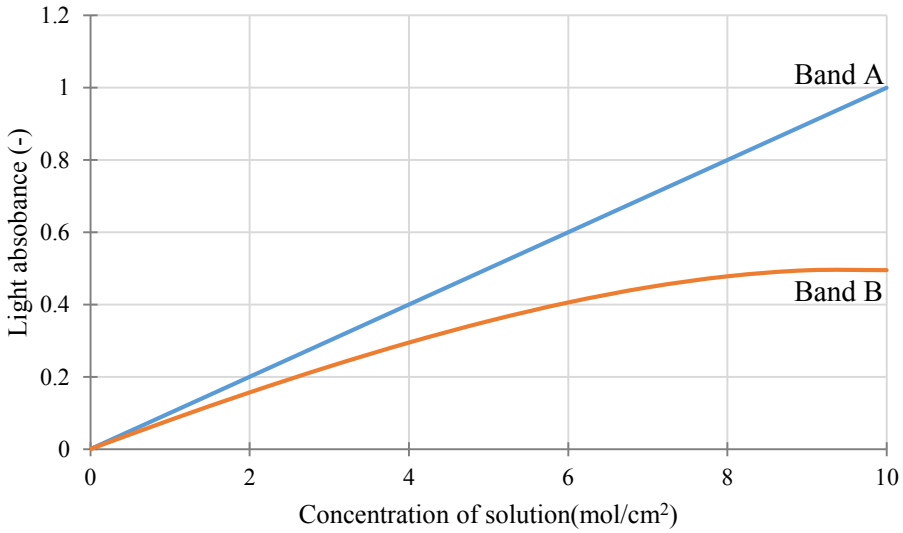


Figure 2.37. Deviated light absorbance of 2 wavelengths.

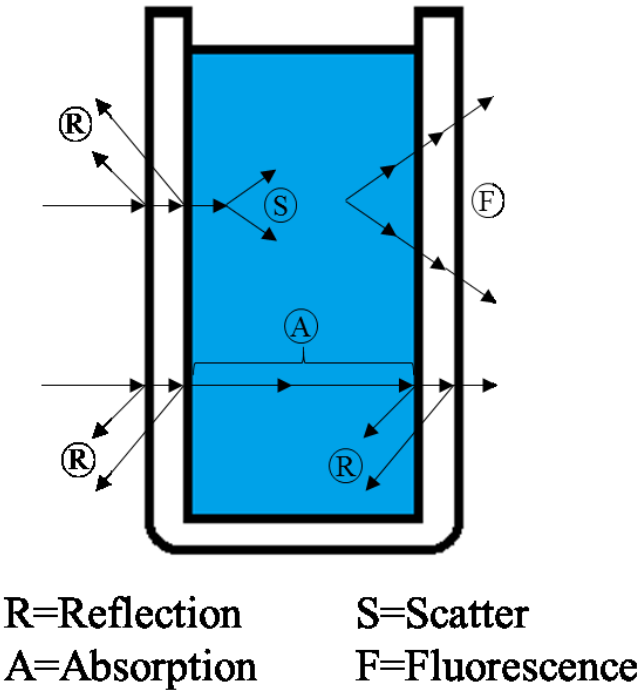


Figure 2.38. Case of stray radiation excepting the case of instrument.

The deviation does not occur only the polychromatic light, it occurs by the stray radiation. The radiation stray is due to reflection and scattering by the surfaces of lenses, mirrors, gratings, filters, or windows in a light source or monochromator. The deviation of the stray radiation does not occur in the instrument only, the stray radiation occurs also in solution and the cell or cuvette. Figure 2.38 shows the case of stray radiation excepting the case of instrument. It occurs following:

1) Reflection of air or cell and solution /cell interfaces
2) Scattering by the any suspended particles of the solution
3) Absorption by solution
4) Fluorescence solution which occurs by the absorption of the energy and emits the energy at the different wavelength

The light absorbance which has error which occurs from air or cell is calculated by (2.79). k is the constant value which is occurred by the phenomenon in the wall of cell.

$$A = \varepsilon cl + k \tag{2.79}$$

3. Suggestion of the novel spectrophotometric method

In the case of the pure solution, the linear regression analysis is preformed to calculate the linear function that is relationship between the concentration and the light absorbance. The linear function is calculated by some known concentration data. In ideal case, the calculation of the linear regression analysis is perfect. The ideal light absorbance is proportional to the concentration of solution shown in figure 3.1. The dash line in the figure 3.1 shows the linear function calculated by the linear regression analysis from some known concentration data in the function of deviation of Beer-Lambert's law. However, in the case of the deviation of Beer-Lambert's law, the light absorbance is not proportional to the concentration. Therefore, there are many errors when the calculated function is compared with the function of deviation of Beer-Lambert's law. The function of the deviation of the Beer-Lambert's law is the non-linear function. To reduce errors, a nonlinear approximation method is provided. This section analyses the nonlinear approximation methods. The proposed spectrophotometric method provides the method that is appropriate with the deviation of Beer-Lambert's law. After that, the proposed method is analysed in the case of the pure solution and the multi-component solution. Further, the design of the fuzzy set is in this section also.

3.1. Analysis of the nonlinear approximation method

The nonlinear approximation method provides some data in the nonlinear function to estimate a value of the nonlinear function. This subsection analyses the nonlinear approximation method that is appropriate with the deviation of Beer-Lambert's law. The analysed methods are included with a linear interpolation and a polynomial regression analysis.

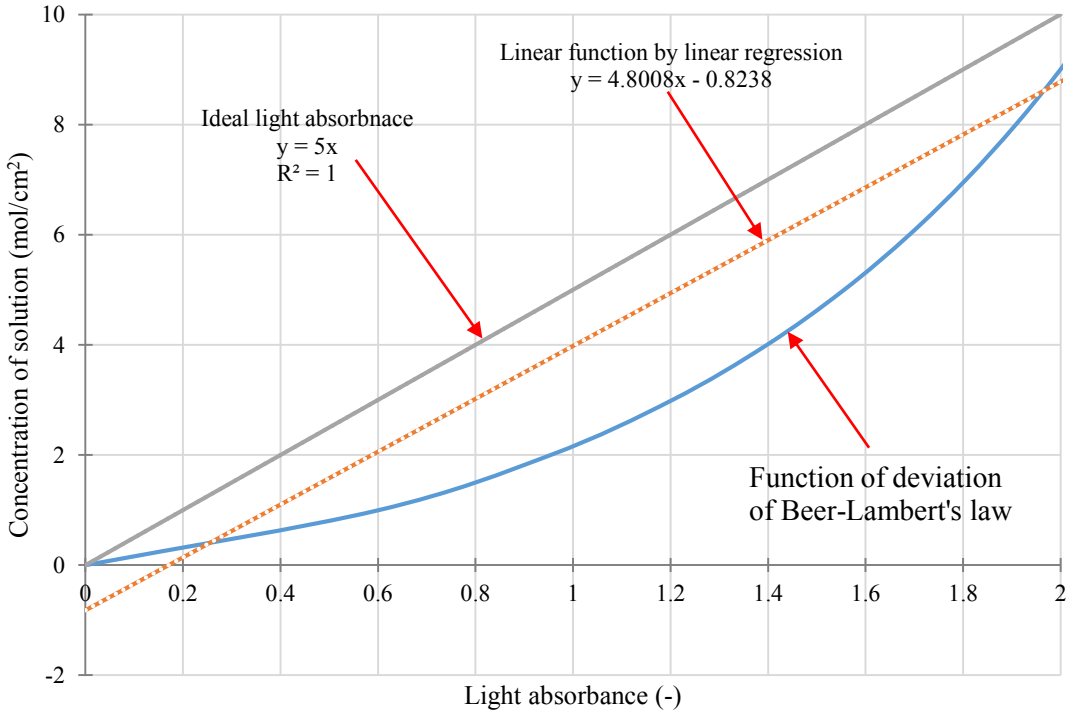


Figure 3.1. Linear function by linear regression analysis.

3.1.1. Polynomial regression analysis

The polynomial regression analysis is a form of the regression analysis. The polynomial regression analysis provides some known concentration data to estimate or predict the data of the nonlinear function [62-63]. In the case of the spectrophotometric method, the polynomial regression analysis calculates the concentration of solution as the polynomial function. The approximation depends on the number of the known concentration data, position of the known concentration data and degree of the polynomial equation. The function is calculated by the polynomial regression analysis shown in (3.1). *n* is the degree of the polynomial equation.

$$f(x) = y = a_0 + a_1 x + a_2 x^2 + \cdots + a_n x^n \tag{3.1}$$

The error (e_i) at each data (x_i, y_i) is calculated by (3.2). *i* is the ordinal number of data. The sum of the squares of all error is shown in (3.3).

$$e_i = y_i - f(x) = y_i - a_0 + a_1 x_i + a_2 x_i^2 + \cdots + a_n x_i^n \tag{3.2}$$

$$S = y_i - f(x) = \sum_{i=1}^{N} (y_i - a_0 + a_1 x_i + a_2 x_i^2 + \cdots + a_n x_i^n) \tag{3.3}$$

To minimize errors, the equation (3.4) is obtained. The errors are minimized in every coefficient case.

$$\begin{aligned} \frac{dS}{da_0} = 0 &= \sum_{i=1}^{N} 2(y_i - a_0 + a_1 x_i + a_2 x_i^2 + \cdots + a_n x_i^n)(-1) \\ \frac{dS}{da_1} = 0 &= \sum_{i=1}^{N} 2(y_i - a_0 + a_1 x_i + a_2 x_i^2 + \cdots + a_n x_i^n)(-x_i) \\ &\vdots \\ \frac{dS}{da_n} = 0 &= \sum_{i=1}^{N} 2(y_i - a_0 + a_1 x_i + a_2 x_i^2 + \cdots + a_n x_i^n)(-x_n) \end{aligned} \tag{3.4}$$

The equations (3.4) is rewritten by equations (3.5).

$$\begin{aligned} a_0 N + a_1 \sum x_i + a_2 \sum x_i^2 + \cdots + a_n \sum x_i^n &= \sum y_i \\ a_0 \sum x_i + a_1 \sum x_i^2 + a_2 \sum x_i^3 + \cdots + a_n \sum x_i^{n+1} &= \sum x_i y_i \\ a_0 \sum x_i^2 + a_1 \sum x_i^3 + a_2 \sum x_i^4 + \cdots + a_n \sum x_i^{n+2} &= \sum x_i^2 y_i \\ &\vdots \\ a_0 \sum x_i^n + a_1 \sum x_i^{n+1} + a_2 \sum x_i^{n+2} + \cdots + a_n \sum x_i^{2n} &= \sum x_i^n y_i \end{aligned} \tag{3.5}$$

The equation (3.5) is rewritten by the matrix equation in (3.6).

$$\begin{bmatrix} N & \sum x_i & \sum x_i^2 & & \sum x_i^n \\ \sum x_i & \sum x_i^2 & \sum x_i^3 & \cdots & \sum x_i^{n+1} \\ \sum x_i^2 & \sum x_i^3 & \sum x_i^4 & & \sum x_i^{n+2} \\ & \vdots & & \ddots & \vdots \\ \sum x_i^n & \sum x_i^{n+1} & \sum x_i^{n+2} & \cdots & \sum x_i^{2n} \end{bmatrix} \begin{bmatrix} a_0 \\ a_1 \\ a_2 \\ \vdots \\ a_n \end{bmatrix} = \begin{bmatrix} \sum y_i \\ \sum x_i y_i \\ \sum x_i^2 y_i \\ \vdots \\ \sum x_i^n y_i \end{bmatrix} \tag{3.6}$$

In the case that the degree of equation (*n*) is 1, the analysis becomes the linear regression analysis shown in (3.7). The equation (3.7) is the same as the linear regression equation in (2.15). To minimize errors, the matrix equation in (3.8) is obtained. The coefficient is calculated by equation (3.9) and (3.10). The equation (3.9) is the same as the equation (2.17) and the equation (3.10) is the same as the equation (2.16).

$$y = a_0 + a_1 x \tag{3.7}$$

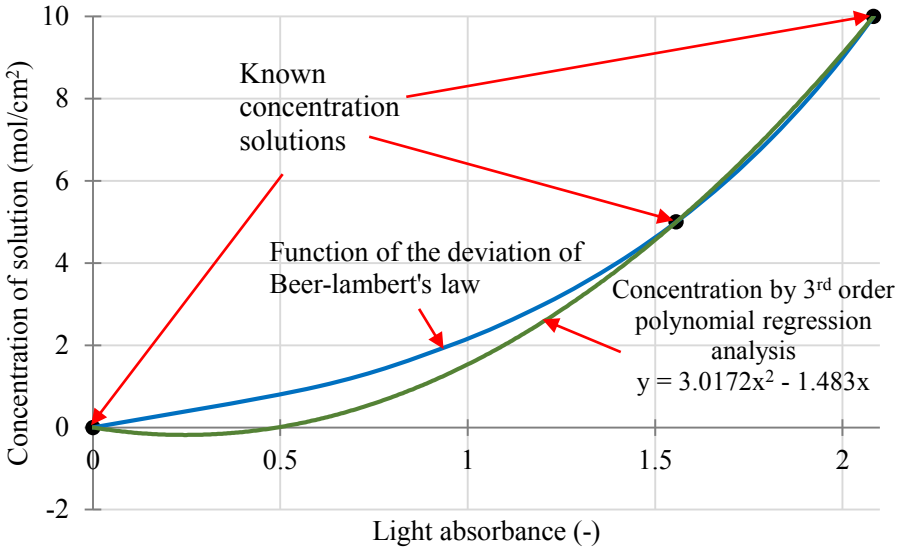


Figure 3.2. Concentration calculated by polynomial regression analysis.

$$\begin{bmatrix} N & \sum x_i \\ \sum x_i & \sum x_i^2 \end{bmatrix} \begin{bmatrix} a_0 \\ a_1 \end{bmatrix} = \begin{bmatrix} \sum y_i \\ \sum x_i y_i \end{bmatrix} \tag{3.8}$$

$$a_0 = \frac{\sum y_i}{N} - a_1 \frac{\sum y_i}{N} \tag{3.9}$$

$$a_1 = \frac{\sum x_i y_i - \frac{\sum x_i y_i}{N}}{\sum x_i^2 - \frac{(\sum x_i)^2}{N}} \tag{3.10}$$

Furthermore, the polynomial regression analysis provides more the number of the known data (N) than the degree of polynomial equation (n). If the number of known data (N) is equal or lower than the degree of polynomial equation (n), the calculated coefficient of the terms that degree is equal or higher than the number of the known data are 0. For an example, if the number of the known concentration solution is 2, the coefficient of the terms that degree is equal and more than 2 are 0. Therefore, the polynomial regression analysis must provide the number of the known data more than 3. Figure 3.2 shows the concentration calculated by the polynomial regression analysis from 3 known concentration solution in function of the deviation function. The calculated concentration is explained by the 2nd order polynomial function.

3.1.2. Linear interpolation

The linear interpolation provides the known concentration data as the boundary points to calculate the concentration in the range of the boundary points [51-52]. The calculated concentration is explained by the linear function in the range of the boundary points shown in figure 3.3. The calculation of the linear interpolation is shown in (3.11).

$$c = \frac{c_0(A_1 - A) + c_1(A - A_0)}{(A_1 - A_0)} \tag{3.11}$$

When many ranges of the boundary points are connected, the calculated concentration is explained by the piecewise linear function shown in (3.12). Figure 3.4 shows the concentration calculated by the linear interpolation from 3 known concentration solution in 2 ranges of the boundary points.

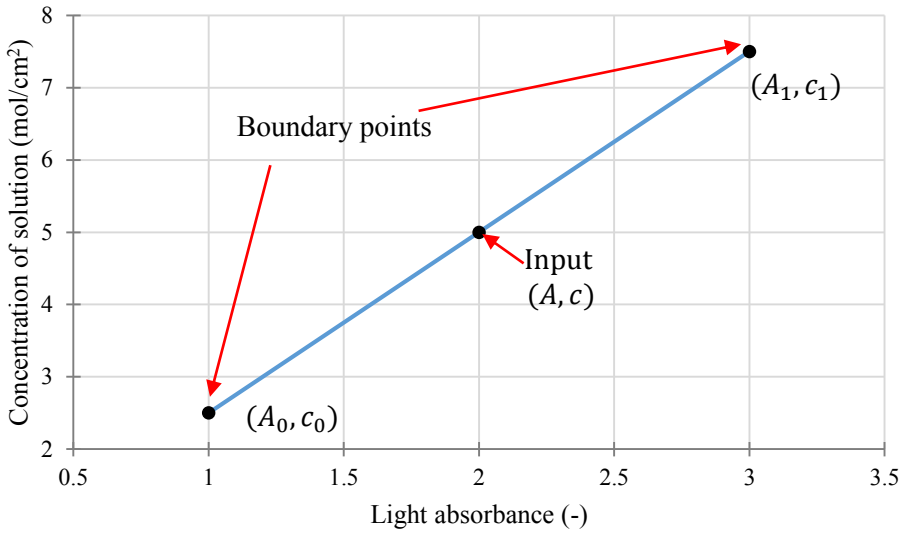


Figure 3.3. Linear interpolation.

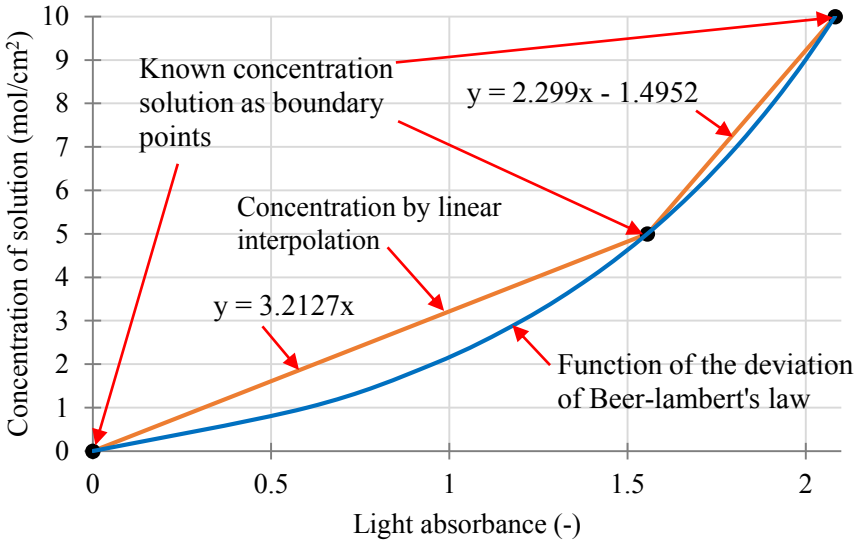


Figure 3.4. Concentration calculated by linear interpolation.

$$c(A) = \begin{cases} \frac{c_0(A_1 - A) + c_1(A - A_0)}{(A_1 - A_0)} & A_0 < A < A_1 \\ \frac{c_1(A_2 - A) + c_2(A - A_1)}{(A_2 - A_1)} & A_1 < A < A_2 \\ \vdots & \\ \frac{c_n(A_{n+1} - A) + c_{n+1}(A - A_n)}{(A_{n+1} - A_n)} & A_{n+1} < A < A_n \end{cases} \tag{3.12}$$

3.1.3. Comparison of the nonlinear approximation

Figure 3.5 shows the concentration calculated by the linear interpolation, the 2nd order polynomial regression from 3 known concentration solutions and a linear regression analysis. The concentration calculated by the nonlinear approximation method is more approximate to the concentration of the deviation of Beer-

Lambert's law than the concentration calculated by the linear regression analysis. It shows that the nonlinear approximation method can reduce errors from the concentration of solution.

In the calculation case, the calculation of the polynomial regression analysis must provide the matrix to calculate the coefficient in the polynomial function shown in (3.6). Therefore, the calculation of the polynomial regression analysis takes more time than the calculation of the linear interpolation that is only linear function in equation (3.11). In the pure solution case, the non-linear approximation is only 1 time. However, in the multi-component case, the calculation of the non-linear approximation requires many rounds to finish. Therefore, the calculation of the polynomial regression analysis takes time too much. In real deviation, the deviation of Beer-Lambert's law occurs at the high concentration. Therefore, the relationship between the light absorbance and the concentration of solution is linear function at the low concentration shown in figure 3.6. The interpolation calculates the concentration as the linear function in the range of the boundary points.

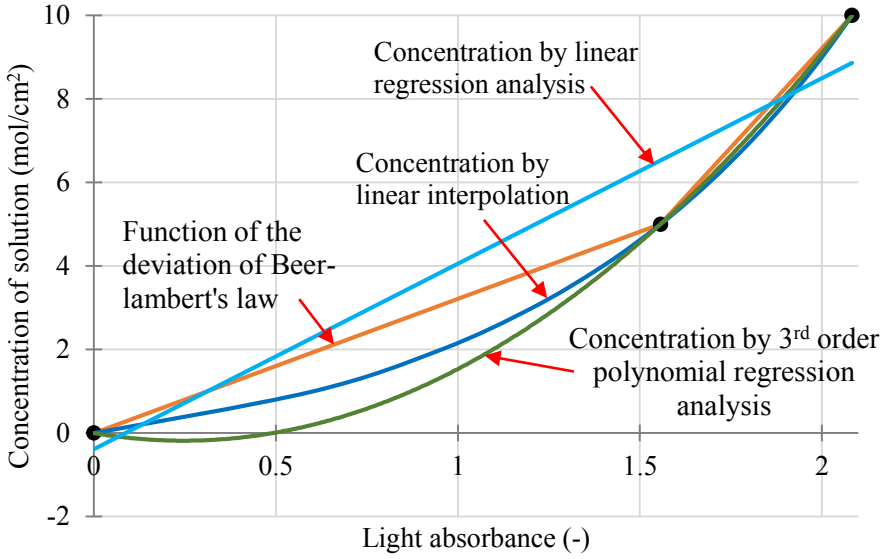


Figure 3.5. Comparison of the nonlinear approximation.

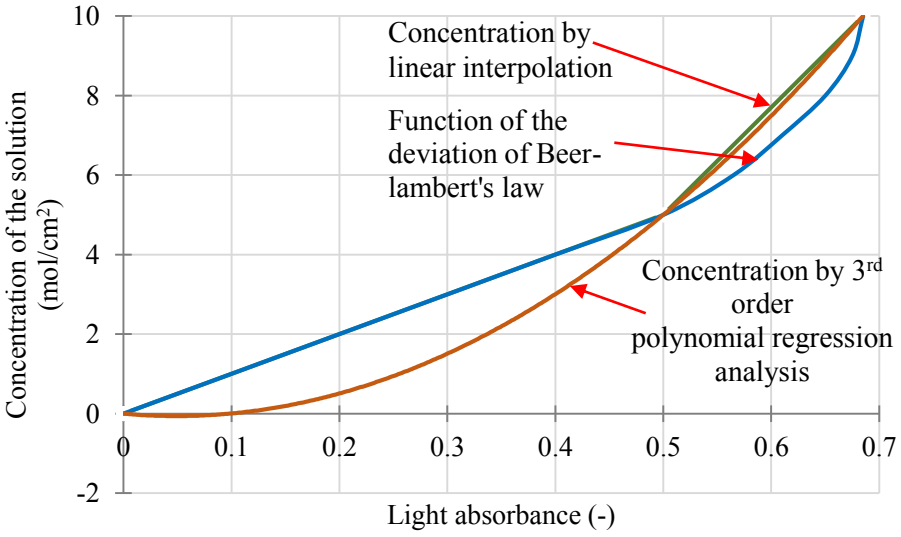


Figure 3.6. Comparison of the nonlinear approximation in real deviation case.

Thus, the error of the concentration calculated by the linear interpolation is very low. However, the polynomial regression analysis calculates the concentration as the polynomial function. As a result, there are very major errors at the low concentration. Moreover, the polynomial regression analysis cannot calculate the concentration in the 2 known concentration solutions case. Hence, the proposed method provides the linear interpolation to calculate the concentration of solution.

3.2. Analysis of the proposed spectrophotometric method in the pure solution

The novel spectrophotometric method is based on the fuzzy theory. The fuzzy theory is explained in this subsection. In classical logic, the truth values of the variable are true (denoted by 1) and false (denoted by 0) only [64-65]. It looks like the decision of concentration by individual light absorbance. It resembles a good calculation. However, in the reality, this method cannot be used because of the unlimited data. Therefore, the decision of every data is impossible. Therefore, the fuzzy theory is developed to solve this problem.

This theory calculates the desire crisp output from the desire crisp input. The desire crisp input is the light absorbance (A) and the desire crisp output is concentration (c). The process of the fuzzy theory to calculate the concentration of solution is exhibited to figure 3.7 [66]. It provides many truth values and the degree of the truth value (W_i) to calculate the desire crisp output. The truth values are values of the known concentration solutions. In the pure solution, there are the light absorbance of the known concentration solution (A_i) and the concentration of known concentration solution (c_i). The truth value of the light absorbance is utilized to design fuzzy set. The membership function in the fuzzy set is degree of the value of the known concentration solution.

The first step of the fuzzy theory is the fuzzification. The fuzzification decides the degree of each function of the known concentration solution (W_i) by the light absorbance (A). The degree of each function of the known concentration solution (W_i) are the input fuzzy value and the linguistic variable which explains the situation of the value. After that, the inference converts the linguistic variable which is the function of the light absorbance of known concentration solution (A_i) to the fuzzy value or crisp value (S_i) by if-then rule as the output fuzzy value. The if-then rule is designed by the truth value which is the concentration of solution (c_i).

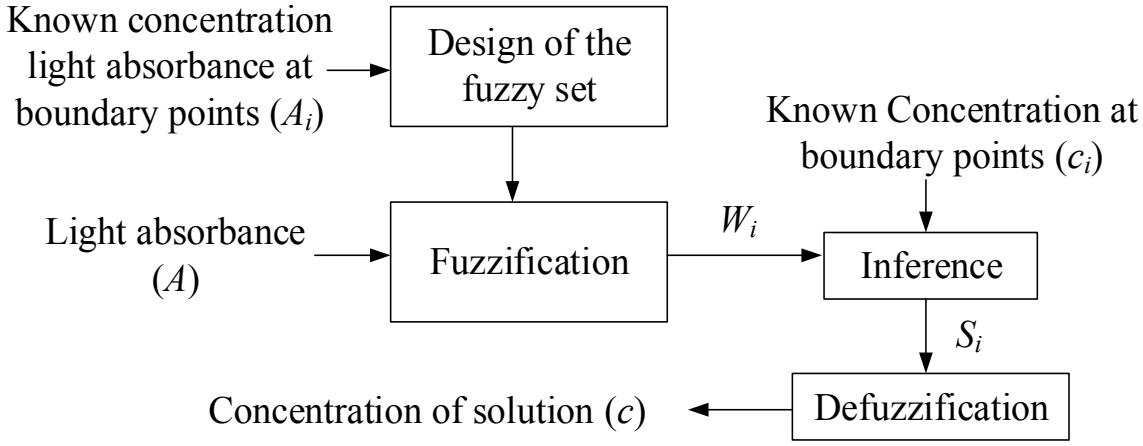


Figure 3.7. Process of the fuzzy theory to calculate the concentration of solution.

If the light absorbance (A) is the known concentration light absorbance (A_i), the concentration of solution (S_i) is the known concentration (c_i).

Last, the defuzzification provides the degree of each function (W_i) and the crisp value from inference process (S_i) to calculate the concentration of solution (c). The defuzzification of this research utilizes the weighted average method or center of gravity [67-71]. The defuzzification equation of weighted average method is shown in equation (3.13).

$$c = \frac{\sum_{i=0}^{k} S_i W_i}{\sum_{i=0}^{k} W_i} \tag{3.13}$$

To design the membership function, the calculation of the proposed method is analysed and compared with the defuzzification equation in (3.13). The calculation of the proposed method is the linear interpolation. The linear interpolation provides the known concentration data as the boundary points to calculate the concentration of solution. The coefficient of the known concentration in equation (3.11) is explained by the linear function in the range of the boundary points shown in figure 3.8. The degree of the coefficient of the known concentration is from 0 to 1. When many ranges of the boundary points are combined, the coefficient of the known concentration is explained by the triangle membership function shown in figure 3.9.

The inference process of the fuzzy theory converts the linguistic value of each function to the crisp value (S_i) by the known concentration (c_i). Therefore, the crisp value (S_i) is equal to the known concentration of solution (c_i). Therefore, the coefficient of the known concentration is provides as the membership function following the linear interpolation equation in (3.11). The sum of coefficient of the known concentration of each function ($\sum_{i=0}^{k} W_i$) is 1. Thus, the denominator of the defuzzification equation is 1 also. The calculation of the defuzzification is the same as the calculation of the linear interpolation. The degree of known concentration is explained by the triangle membership function same as the coefficient of the known concentration.

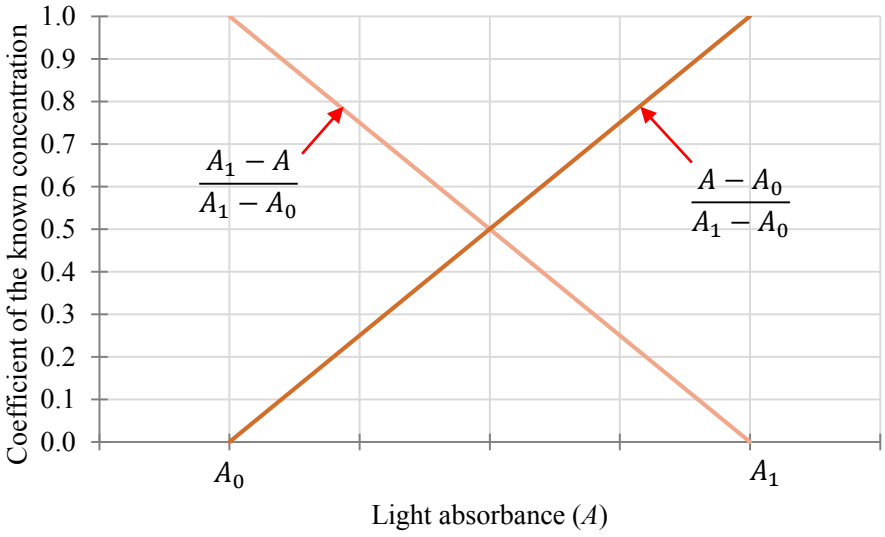


Figure 3.8. Coefficient of the known concentration.

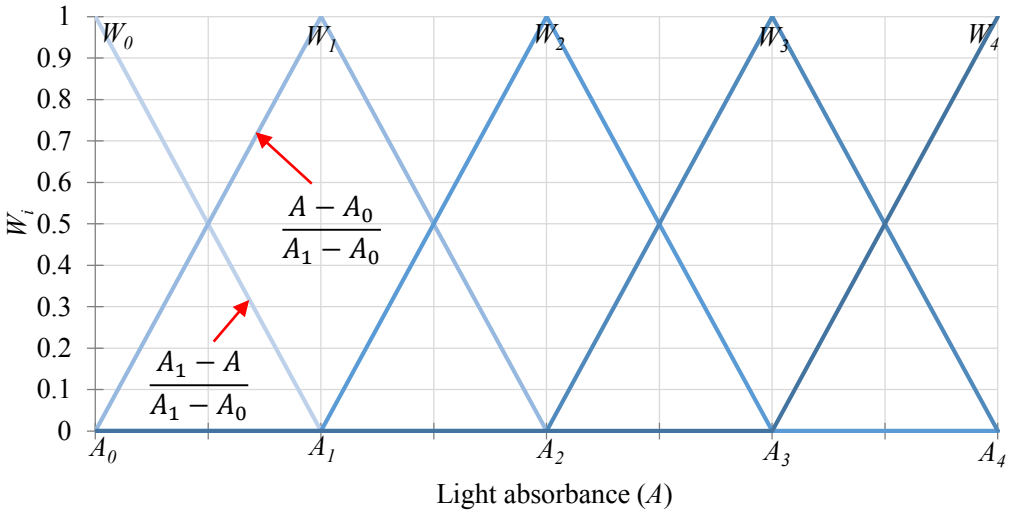


Figure 3.9. Fuzzy set in the case of the pure solution.

3.3. Analysis of the proposed spectrophotometric method in the multi-component solution

In this sub-section, the exhibition of the proposed spectrophotometric method is performed in 2-component solution case. From the previous multicomponent spectrophotometric methods, they are categorized into 2 main methods. The 1st method provides all molar absorptivity and light absorbance. The 2nd method is the elimination of the noise of the disinterest component. The calculation by fuzzy theory is similar to the simultaneous equation.

The calculation of proposed method in the case of the 2-component case is similar to the bilinear interpolation. The bilinear interpolation averages the data in the area of the boundary points shown in figure 3.10. The bilinear interpolation starts to calculate the linear interpolation direction by direction. The bilinear interpolation has 2 inputs. Therefore, there are 2 processes. The first process is the linear interpolation in direction of one input. The second process is the linear interpolation in direction of another input. For an example, in figure 3.10, it starts to calculate the linear interpolation in the x-direction. The data is interpolated at point (x, y_0) and (x, y_1). The calculation equations of 2 points are shown in (3.14) and (3.15).

$$f(x, y_0) = \frac{x_1 - x}{x_1 - x_0} f(x_0, y_0) + \frac{x - x_0}{x_1 - x_0} f(x_1, y_0) \tag{3.14}$$

$$f(x, y_1) = \frac{x_1 - x}{x_1 - x_0} f(x_0, y_1) + \frac{x - x_0}{x_1 - x_0} f(x_1, y_1) \tag{3.15}$$

Next process is the interpolation in y-direction. The linear interpolation in 2nd process provides the data at point (x, y_0) and (x, y_1). The data at point (x, y) is interpolated by equation (3.16).

$$f(x, y) = \frac{y_1 - y}{y_1 - y_0} f(x, y_0) + \frac{y - y_0}{y_1 - y_0} f(x, y_1) \tag{3.16}$$

The contour graph of the bilinear interpolation in figure 3.11 shows that the direction of the range of the boundary points is the same as the direction of the input. However, in the case of the spectrophotometric method, the direction of range of the boundary points which is the direction of the component is not the same as the direction of the light absorbance shown in figure 3.12.

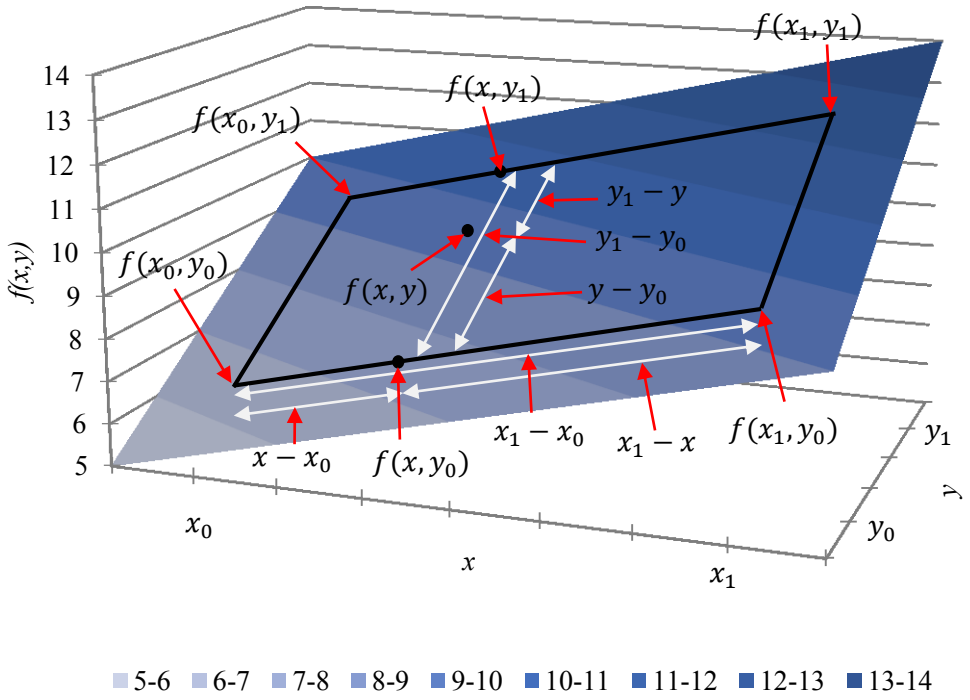


Figure 3.10. Bilinear interpolation.

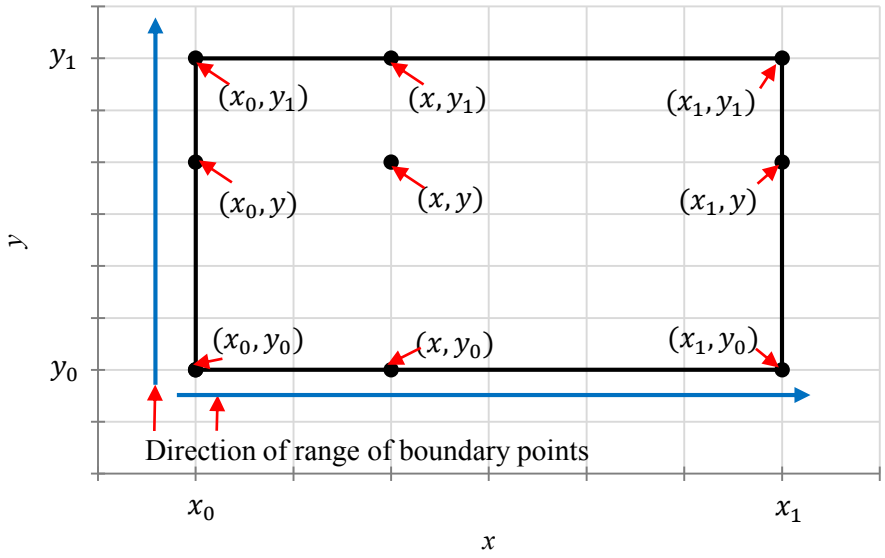


Figure 3.11. Bilinear interpolation (contour graph).

The boundary points are set by the ideal light absorbance of the 1st detector and the 2nd detector in any level of the concentration of 2 components in table 3.1 and 3.2. Therefore, one boundary point has 4 variables that are light absorbance of 1st detector and 2nd detector and the concentration of the 1st component and 2nd component in one solution.

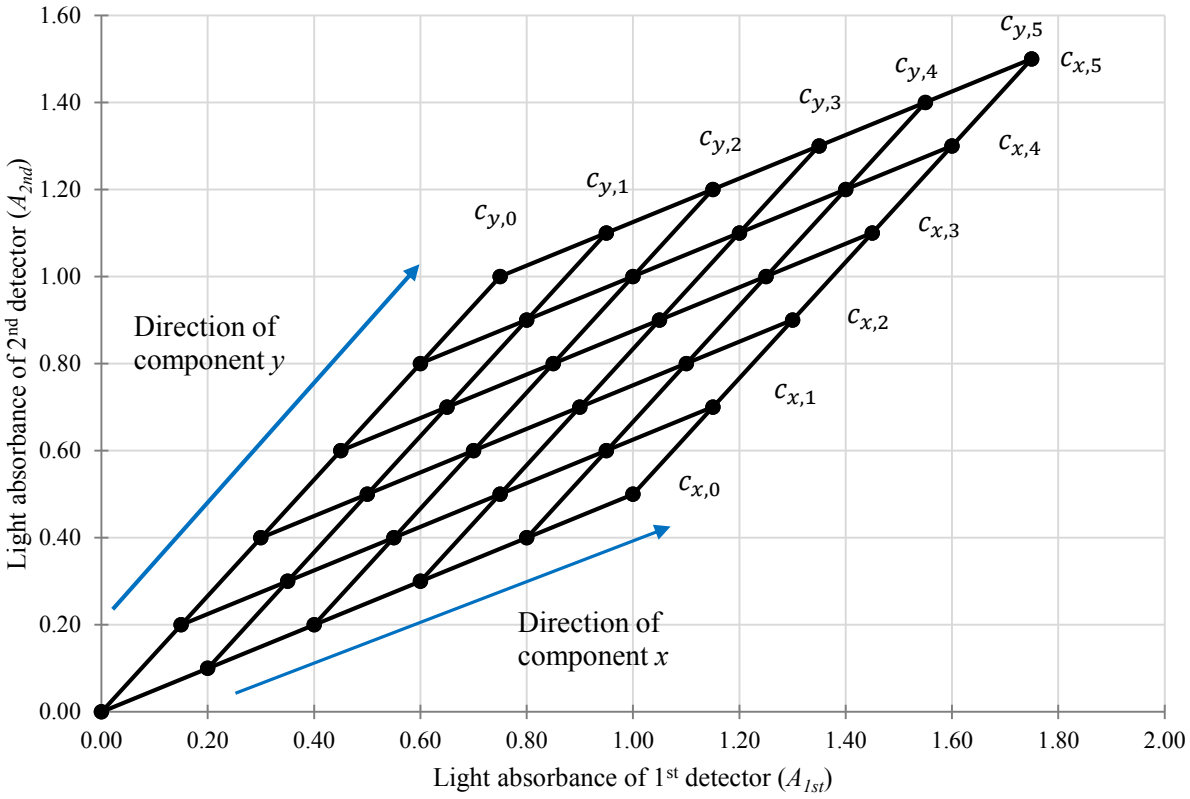


Figure 3.12. Boundary points of the mixture solution (contour graph).

Table 3.1. Ideal known concentration light absorbance of the 1st detector ($A_{i,j,1st}$).

	***y*0**	***y*1**	***y*2**	***y*3**	***y*4**	***y*5**
***x*0**	0.00	0.20	0.40	0.60	0.80	1.00
***x*1**	0.15	0.35	0.55	0.75	0.95	1.15
***x*2**	0.30	0.50	0.70	0.90	1.10	1.30
***x*3**	0.45	0.65	0.85	1.05	1.25	1.45
***x*4**	0.60	0.80	1.00	1.20	1.40	1.60
***x*5**	0.75	0.95	1.15	1.35	1.55	1.75

Table 3.2. Ideal known concentration light absorbance of the 2nd detector ($A_{i,j,2nd}$).

	***y*0**	***y*1**	***y*2**	***y*3**	***y*4**	***y*5**
***x*0**	0	0.1	0.2	0.3	0.4	0.5
***x*1**	0.2	0.3	0.4	0.5	0.6	0.7
***x*2**	0.4	0.5	0.6	0.7	0.8	0.9
***x*3**	0.6	0.7	0.8	0.9	1	1.1
***x*4**	0.8	0.9	1	1.1	1.2	1.3
***x*5**	1	1.1	1.2	1.3	1.4	1.5

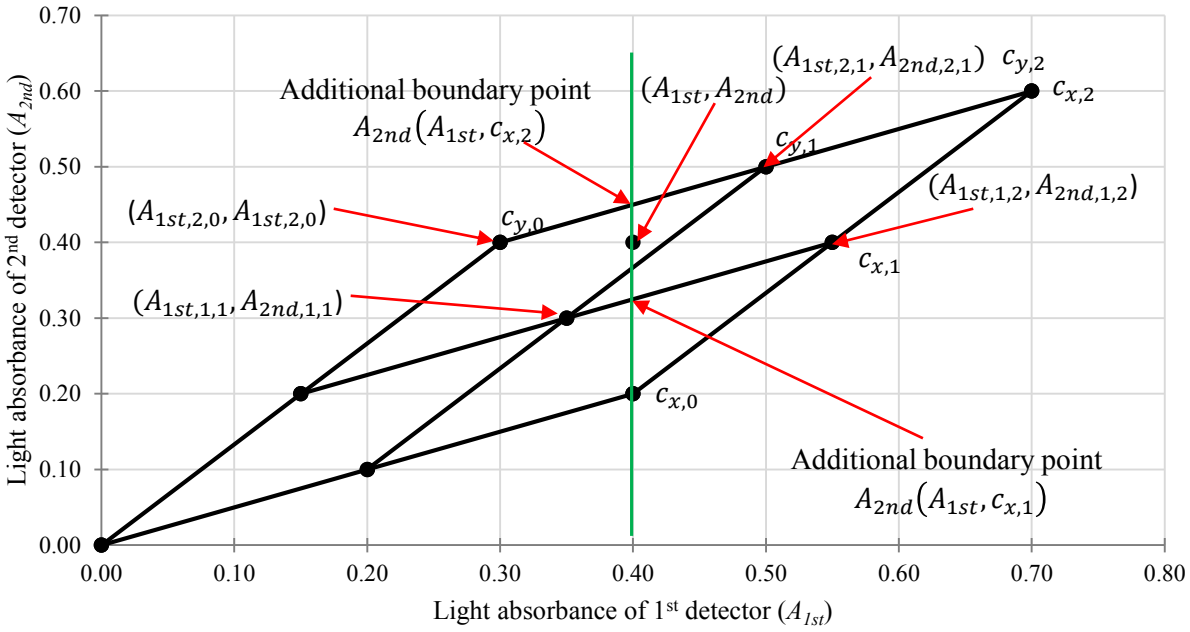


Figure 3.13. Calculation of the additional boundary points (contour graph).

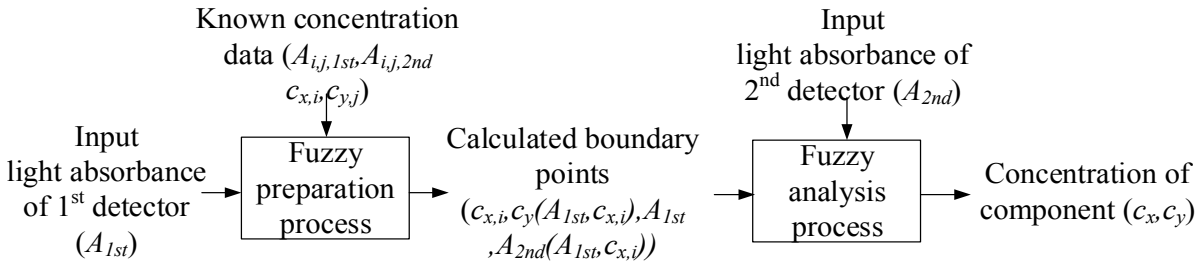


Figure 3.14. Process of the proposed method in 2-component solution case.

In the fuzzy theory, the interpolation employs the triangle membership function. Therefore, in the case of the bilinear interpolation, the pyramid membership function [72]. However, the interpolation area of the mixture solution is the trapezium shape shown that in figure 3.12. It is difficult to calculate degree of the pyramid membership function based on trapezium shape. Therefore, the calculation of the concentration of the proposed method is separated to 2 processes same as the bilinear interpolation. The membership functions of the 2 processes are the triangle membership function. As the direction of the component is not the same as the direction of the light absorbance, there is an additional calculation of the boundary points in the direction of the one concentration by the light absorbance of one detector shown in figure 3.13. After that, the concentrations at each addition boundary point are provided to interpolate the concentration of solution in direction of the light absorbance of another detector by the light absorbance of another detector. The process of the proposed method is shown in figure 3.14. There are fuzzy preparation process and the fuzzy analysis process. The boundary points in the function of the concentration of component *x* are shown in figure 3.15 and the boundary points in the function of the concentration of component *y* are shown in figure 3.16.

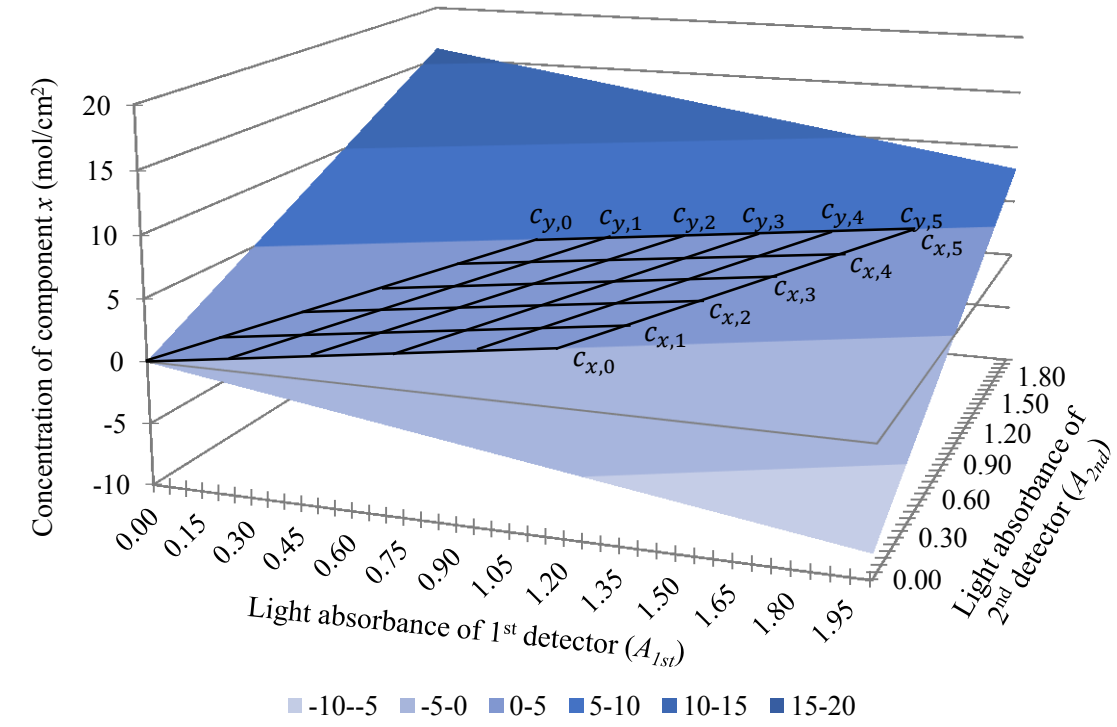


Figure 3.15. Boundary points in the function of the concentration of component *x*.

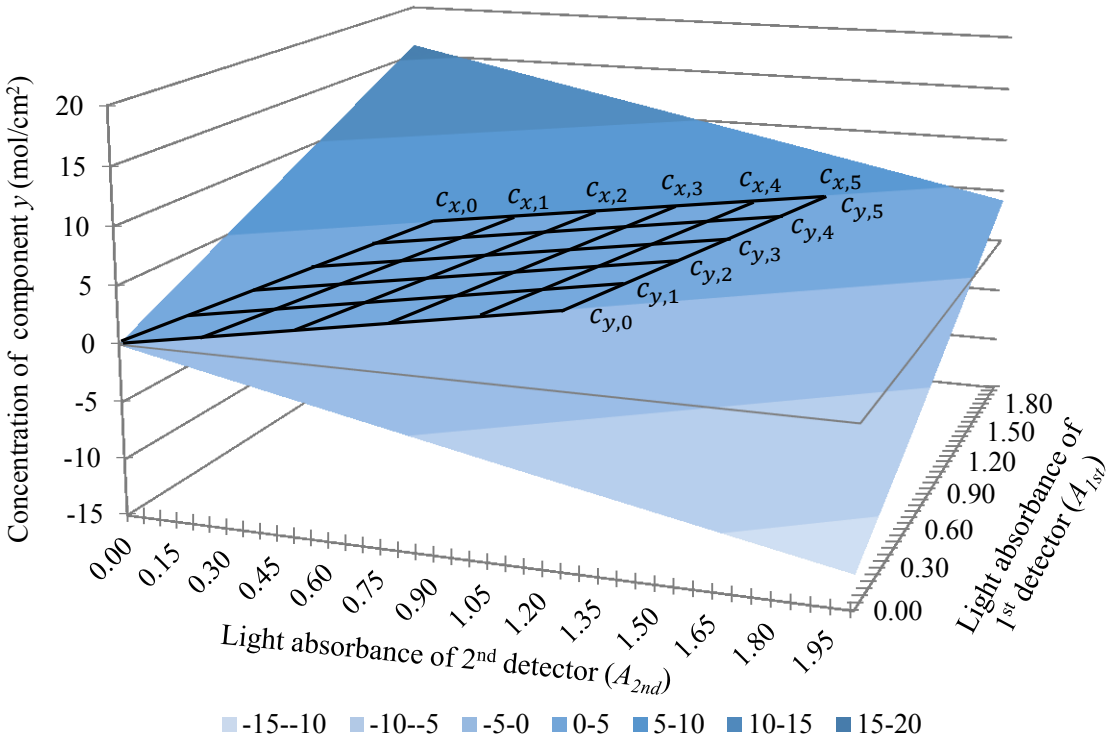


Figure 3.16. Boundary points in the function of the concentration of component *y*.

3.3.1. Fuzzy preparation process

The fuzzy preparation process interpolates the value of 4 variables at the additional boundary points by the value of the variables at boundary points. To calculate the value of the 4 variables, the relationship between the 4 variables is analyzed. In general, the light absorbance is calculated by concentration of all solutions. The light absorbance of the 1st detector (A_{1st}) and the 2nd detector (A_{2nd}) is calculated by the concentration of component *x* (c_x) and the component *y* (c_y) shown in (3.17) and (3.18), respectively.

$$A_{1st} = a_{x_{1st}} c_x + a_{y_{1st}} c_y \tag{3.17}$$

$$A_{2nd} = a_{x_{2nd}} c_x + a_{y_{2nd}} c_y \tag{3.18}$$

The equation (3.17) and (3.18) are rewritten by (3.19) and (3.20). They show that the concentration of component *x* is proportional to the light absorbance of the 1st detector and the 2nd detector when the concentration of the component *y* is fixed.

$$c_x = \frac{A_{1st} - a_{y_{1st}} c_y}{a_{x_{1st}}} \tag{3.19}$$

$$c_x = \frac{A_{2nd} - a_{y_{2nd}} c_y}{a_{x_{2nd}}} \tag{3.20}$$

The equation (3.17) and (3.18) are rewritten by (3.21) and (3.22). They show that the concentration of component *y* is proportional to the light absorbance of the 1st detector and the 2nd detector when the concentration of the component *x* is fixed.

$$c_y = \frac{A_{1st} - a_{x_{1st}} c_x}{a_{y_{1st}}} \tag{3.21}$$

$$c_y = \frac{A_{2nd} - a_{x_{2nd}} c_x}{a_{y_{2nd}}} \tag{3.22}$$

After that, the equation (3.22) is substituted into (3.17). Equation (3.23) is obtained. Furthermore, the equation (3.20) is substituted into (3.17). Equation (3.24) is obtained. They show that the light absorbance of the 1st detector is proportional to the light absorbance of the 2nd detector when the concentrations of the component *x* or component *y* are fixed.

$$A_{1st} = \frac{a_{y_{1st}}}{a_{y_{2nd}}} A_{2nd} - \frac{\left(a_{x_{1st}} a_{y_{2nd}} - a_{x_{2nd}} a_{y_{1st}}\right)}{a_{y_{2nd}}} c_x \tag{3.23}$$

$$A_{1st} = \frac{a_{x_{1st}}}{a_{x_{2nd}}} A_{2nd} - \frac{\left(a_{x_{1st}} a_{y_{2nd}} - a_{x_{2nd}} a_{y_{1st}}\right)}{a_{x_{2nd}}} c_y \tag{3.24}$$

In other words, the equation (3.21) is substituted into (3.18). Equation (3.25) is obtained. Furthermore, the equation (3.19) is substituted into (3.18). Equation (3.26) is obtained. They show that the light absorbance of the 2nd detector is proportional to the light absorbance of the 1st detector when the concentrations of the component *x* or component *y* are fixed.

$$A_{2nd} = \frac{a_{y_{2nd}}}{a_{y_{1st}}} A_{1st} + \frac{\left(a_{x_{1st}} a_{y_{2nd}} - a_{x_{2nd}} a_{y_{1st}}\right)}{a_{y_{1st}}} c_x \tag{3.25}$$

$$A_{2nd} = \frac{a_{x_{2nd}}}{a_{x_{1st}}} A_{1st} + \frac{\left(a_{x_{1st}} a_{y_{2nd}} - a_{x_{2nd}} a_{y_{1st}}\right)}{a_{x_{1st}}} c_y \tag{3.26}$$

For above equations, they show that all variables of one boundary point can be calculated by one light absorbance and fixing of concentration of one concentration. Therefore, the additional boundary points are interpolated by one light absorbance in direction of one solution. $A_{1st,i,j}$ is the light absorbance of the 1st concentration when the concentration of component *x* is *i* and the concentration of component *y* is *j*. $A_{2nd,i,j}$ is the light absorbance of the 2nd concentration when the concentration of component *x* is *i* and the concentration of component *y* is *j*.

For an example, the calculation starts in the direction of the component *x* by the light absorbance of 1st detector. The additional boundary points are calculated at $(A_{1st}, c_{x,1})$ and $(A_{1st}, c_{x,2})$. The light absorbance of the 2nd detector at the additional boundary points are calculated by the equation (3.27) and (3.28) shown in figure 3.13.

$$A_{2nd}(A_{1st}, c_{x,1}) = \frac{(A_{1st,1,2} - A_{1st}) A_{2nd,1,2}}{(A_{1st,1,2} - A_{1st,1,1})} + \frac{(A_{1st} - A_{1st,1,1}) A_{2nd,1,1}}{(A_{1st,1,2} - A_{1st,1,1})} \tag{3.27}$$

$$A_{2nd}(A_{1st}, c_{x,2}) = \frac{(A_{1st,2,1} - A_{1st}) A_{2nd,2,1}}{(A_{1st,2,1} - A_{1st,2,0})} + \frac{(A_{1st} - A_{1st,2,0}) A_{2nd,2,1}}{(A_{1st,2,1} - A_{1st,2,0})} \tag{3.28}$$

As the calculation in the direction of the component *x*, the concentration of the component *x* is fixed in any level shown in figure 3.17. The concentration of the component *x* at the additional boundary points is shown in (3.29) and (3.30).

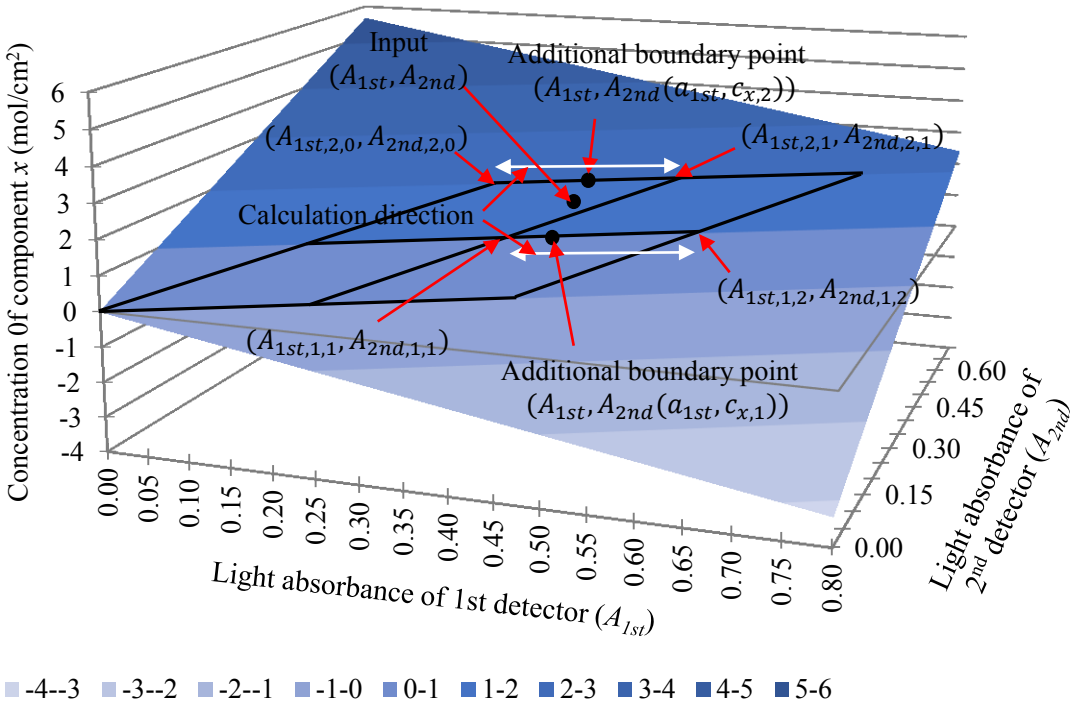


Figure 3.17. Calculation of the concentration of component *x* at additional boundary points.

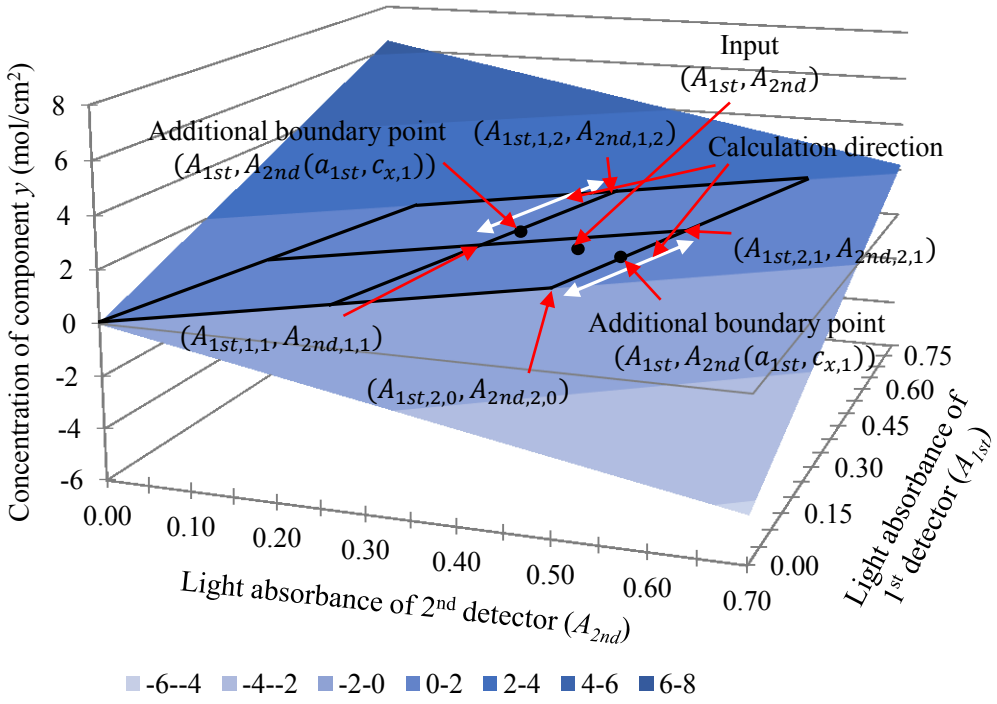


Figure 3.18. Calculation of the concentration of component *y* at additional boundary points.

$$c_x(A_{1st}, c_{x,1}) = c_{x,1} \tag{3.29}$$

$$c_x(A_{1st}, c_{x,2}) = c_{x,2} \tag{3.30}$$

The calculation of concentration the component *y* at additional boundary points is shown in figure 3.18. The concentration of the component *y* at the additional boundary points is calculated by (3.31) and (3.32).

$$c_y(A_{1st}, c_{x,1}) = \frac{(A_{1st,1,2} - A_{1st})c_{y,1}}{(A_{1st,1,2} - A_{1st,1,1})} + \frac{(A_{1st} - A_{1st,1,1})c_{y,2}}{(A_{1st,1,2} - A_{1st,1,1})} \tag{3.31}$$

$$c_y(A_{1st}, c_{x,2}) = \frac{(A_{1st,2,1} - A_{1st})c_{y,0}}{(A_{1st,2,1} - A_{1st,2,0})} + \frac{(A_{1st} - A_{1st,2,0})c_{y,1}}{(A_{1st,1,1} - A_{1st,2,0})} \tag{3.32}$$

The flow of the fuzzy preparation process is shown in figure 3.19. It is same as the pure solution case. The known concentration light absorbance of the 1st detector is provided to design the fuzzy set shown in figure 3.20 that the interpolation is in direction of the concentration is fixed at 1 of component *x*. The fuzzification process provides the light absorbance of the 1st detector to decide the degree of each function ($W_{i,j,1st}$). The ordinary subscript of the degree is the order of process. The 1st subscript is the preparation process. *i* and *j* are concentration of component *x* and component *y*, respectively. After that, the inference converts the linguistic variable which is the function of the known concentration light absorbance of 1st detector ($A_{1st,i,j}$) to the fuzzy value or crisp value that there are crisp value ($S_{2nd,i,j}$) from the light absorbance of known concentration the 2nd detector ($S_{2nd,i,j}$) and crisp value ($S_{y,j}$) from the known concentration of component *y* by if-then rule.

If the light absorbance of the 1st detector (A_{1st}) is the known concentration light absorbance of the 1st detector ($A_{1st,i,j}$), the light absorbance of the 2nd detector at boundary points (i,j) ($S_{2nd,i,j}$) is the light absorbance of known concentration of the 2nd detector ($A_{2nd,i,j}$) and the concentration of component y at boundary points (i,j) ($S_{y,j}$) is the known concentration of the component y ($c_{y,j}$).

The last process of the fuzzy preparation process is the defuzzification. The defuzzification provides the degree of each function ($W_{i,j,1st}$) and the crisp value from inference process which there are the light absorbance of the 2nd detector ($S_{2nd,i,j}$) and the concentration of component y ($S_{y,j}$) at boundary points (i,j). The calculation of the concentration of the component y and the light absorbance of the 2nd detector at additional boundary points ($A_{1st}, c_{x,i}$) are shown in (3.33) and (3.34), respectively.

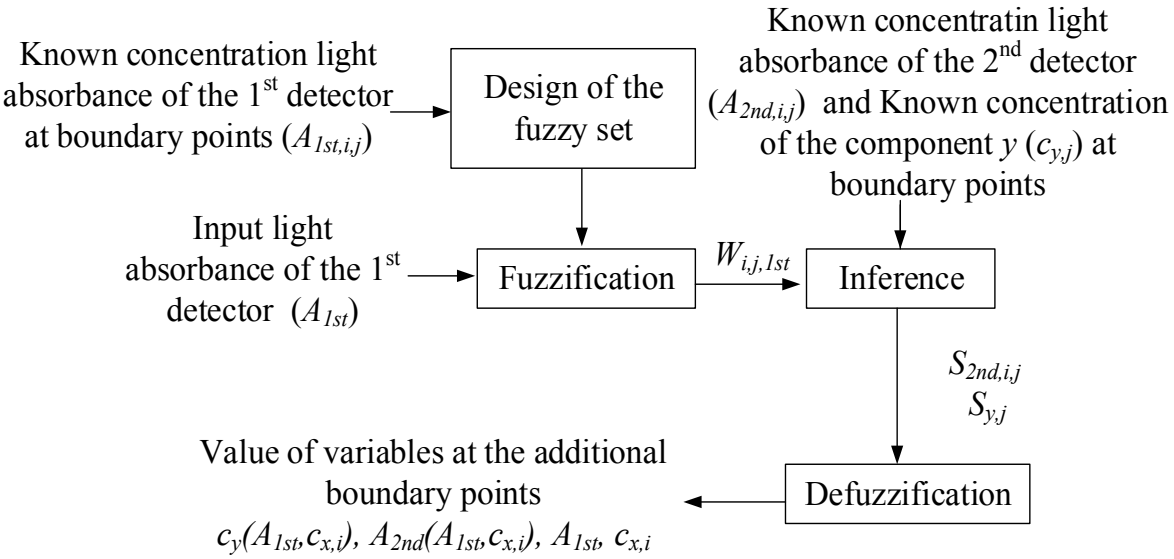


Figure 3.19. Flow of the fuzzy preparation process.

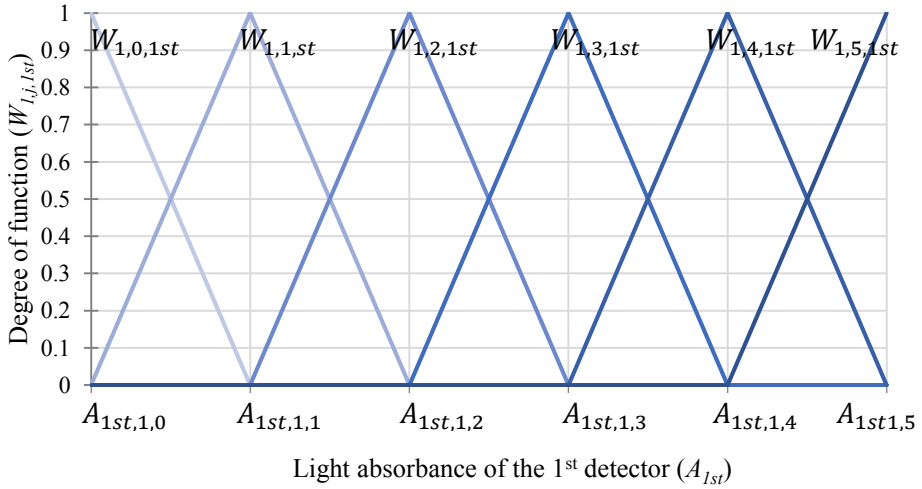


Figure 3.20. Fuzzy set of the fuzzy preparation process.

$$c_y(A_{1st}, c_{x,i}) = \frac{\sum_{j=0}^{k} S_{y,j} W_{i,j,1st}}{\sum_{j=0}^{k} W_{i,j,1st}} \tag{3.33}$$

$$A_{2nd}(A_{1st}, c_{x,i}) = \frac{\sum_{j=0}^{k} S_{2nd,i,j} W_{i,j,1st}}{\sum_{j=0}^{k} W_{i,j,1st}} \tag{3.34}$$

Figure 3.21 shows the additional boundary points calculated in the contour graph by the fuzzy preparation method that the calculation is in the direction of component *x* by the light absorbance of the 1st detector. Table 3.3 shows values of the 4 variables in the additional boundary points in the figure 3.21. It shows that when the light absorbance of the 2nd detector increases, the concentration of component *x* increases and the concentration of component *y* reduces.

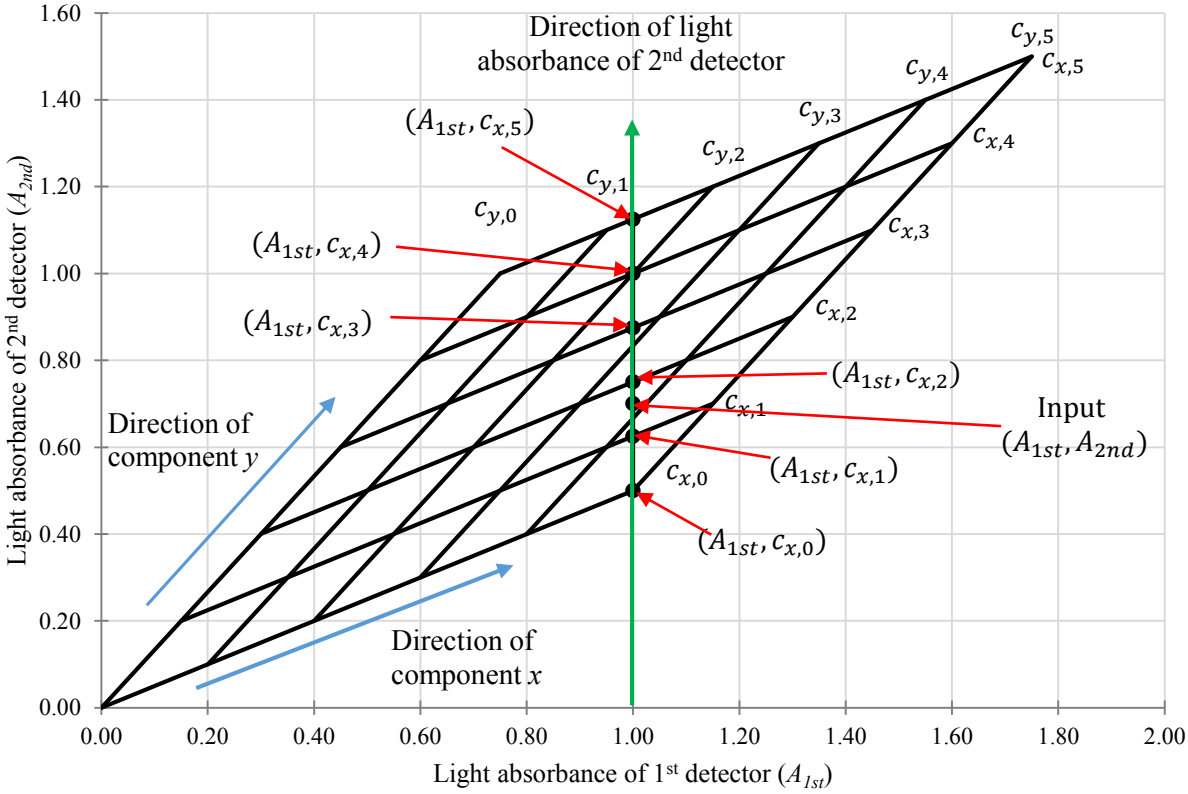


Figure 3.21. Additional boundary points calculated by the fuzzy preparation process (contour graph).

Table 3.3. Value of the variables in additional boundary points.

Boundary point	A_{1st}	$c_y(A_{1st}, c_{x,i})$	$c_{x,i}$	$A_{2nd}(A_{1st}, c_{x,i})$
$(A_{1st}, c_{x,0})$	1	0.5	0	5
$(A_{1st}, c_{x,1})$	1	0.625	1	4..25
$(A_{1st}, c_{x,2})$	1	0.75	2	3.5
$(A_{1st}, c_{x,3})$	1	0.875	3	2.75
$(A_{1st}, c_{x,4})$	1	1	4	2
$(A_{1st}, c_{x,5})$	1	1.125	5	1.25

3.3.2. Fuzzy analysis process

The last process of the proposed method is the fuzzy analysis process. This process interpolates the concentration of component *x* and component *y* in the range of the additional boundary points from the fuzzy preparation process by another inputs. After the fuzzy preparation process in figure 3.14, 3.17 and 3.18, the linear interpolation is performed in direction of the light absorbance of the 2nd detector by the light absorbance of the 2nd detector. The direction of the range of additional boundary points is the same as the direction of the input. The calculation of the component *x* is shown in figure 3.22 and the calculation of the component *y* is shown in figure 3.23. The concentration of component *x* and component *y* are calculated by (3.35) and (3.36), respectively.

$$c_x(A_{1st}, A_{2nd}) = \frac{(A_{2nd}(A_{1st}, c_{x,2}) - A_{2nd})c_{x,1}}{(A_{2nd}(A_{1st}, c_{x,2}) - A_{2nd}(A_{1st}, c_{x,1}))} + \frac{(A_{2nd} - A_{2nd}(A_{1st}, c_{x,1}))c_{x,2}}{(A_{2nd}(A_{1st}, c_{x,2}) - A_{2nd}(A_{1st}, c_{x,1}))} \quad (3.35)$$

$$c_y(A_{1st}, A_{2nd}) = \frac{(A_{2nd}(A_{1st}, c_{x,2}) - A_{2nd})c_y(A_{1st}, c_{x,1})}{(A_{2nd}(A_{1st}, c_{x,2}) - A_{2nd}(A_{1st}, c_{x,1}))} + \frac{(A_{2nd} - A_{2nd}(A_{1st}, c_{x,1}))c_y(A_{1st}, c_{x,2})}{(A_{2nd}(A_{1st}, c_{x,2}) - A_{2nd}(A_{1st}, c_{x,1}))} \quad (3.36)$$

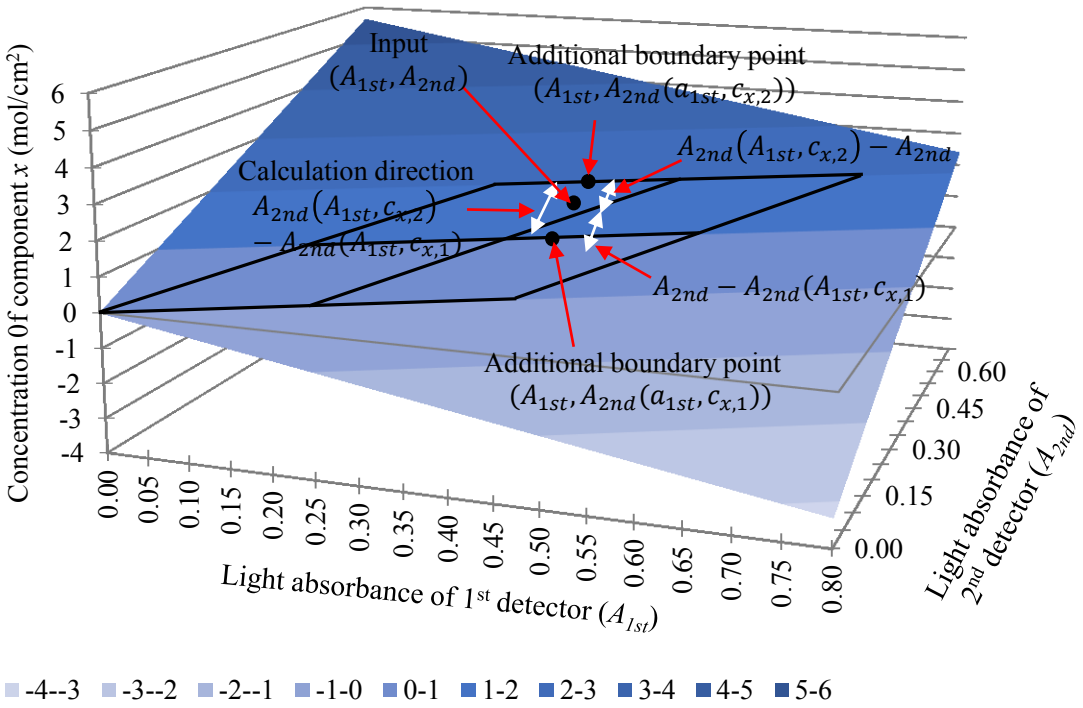


Figure 3.22. Calculation of the concentration of component *x*.

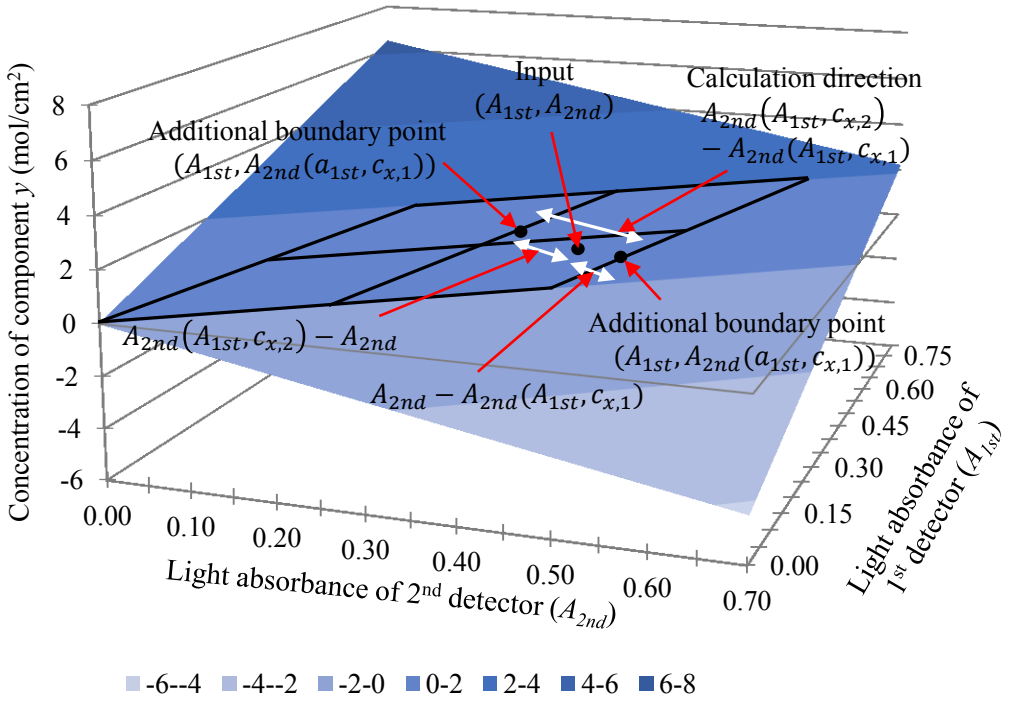


Figure 3.23. Calculation of the concentration of component *y*.

The flow of the fuzzy analysis process is shown in figure 3.24. The light absorbance of the 2nd detector at the additional boundary points is provided to design the fuzzy set shown in 3.25. The fuzzification decides the degree of each function ($W_{j,2nd}$) by the light absorbance of the 2nd detector (A_{2nd}). The 2nd subscript is the analysis process. After that, the inference process converts the linguistic variable which is the function of the light absorbance of 2nd detector at the additional boundary points ($A_{2nd}(A_{1st}, c_{x,i})$) to the fuzzy value or crisp value that there are crisp value ($S_{x,i}$) from the concentration of component *x* at additional boundary points ($c_{x,i}$) and crisp value ($S_{y,i}$) from the concentration of component *y* at additional boundary points ($c_y(A_{1st}, c_{x,i})$) by if-then rule.

If the light absorbance of the 2nd detector (A_{2nd}) is the light absorbance of the 2nd detector at the additional boundary points ($A_{2nd}(A_{1st}, c_{x,i})$), then the concentration of the component *x* ($S_{x,i}$) is the concentration of the component *x* at additional boundary points ($c_{x,i}$) and the concentration of component *y* ($S_{y,i}$) is the concentration of the component *y* at additional boundary points ($c_y(A_{1st}, c_{x,i})$).

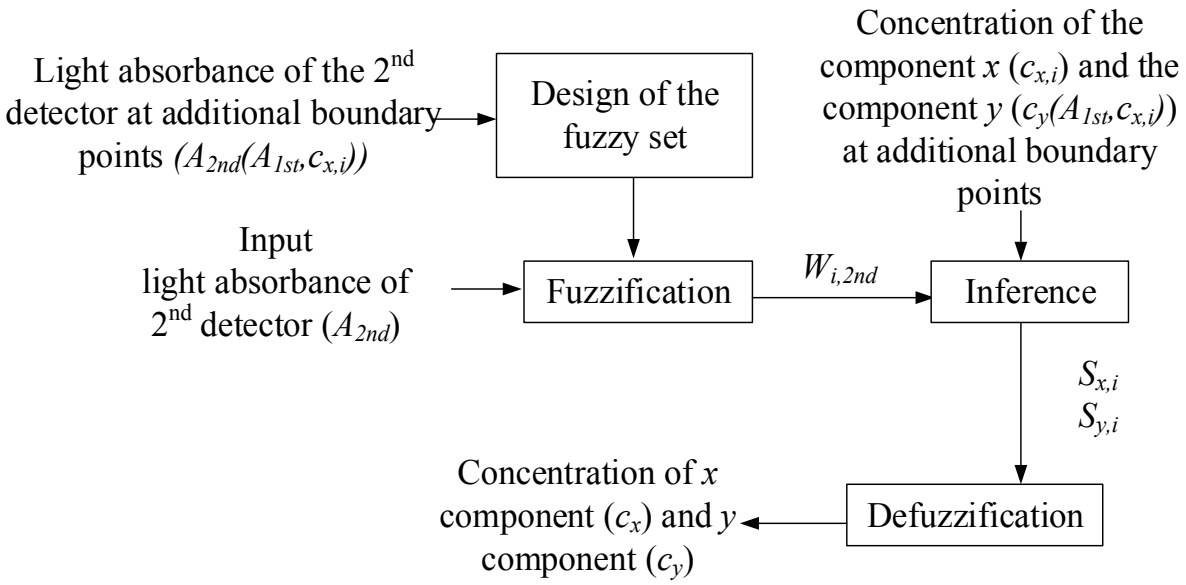


Figure 3.24. Flow of the fuzzy analysis process.

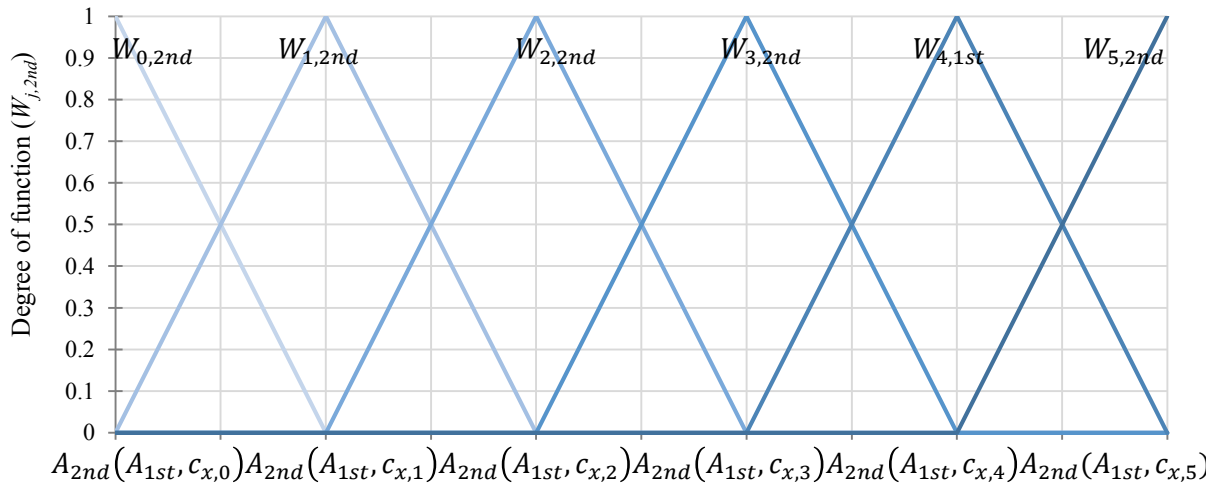


Figure 3.25. Fuzzy set of the fuzzy analysis process.

The last process of the fuzzy analysis process is the defuzzification. The defuzzification provides the degree of each function $(W_{i,2nd})$ and the crisp value from inference process which there are the concentration of the component x $(S_{x,i})$ and the concentration of component y $(S_{y,i})$ at additional boundary points $(A_{1st},c_{x,i})$. The calculation of the concentration of the component x and the component y is shown in (3.37) and (3.38), respectively.

$$c_x(A_{1st}, A_{2nd}) = \frac{\sum_{i=0}^{k} S_{x,i} W_{i,2nd}}{\sum_{i=0}^{k} W_{i,2nd}} \quad (3.37)$$

$$c_y(A_{1st}, A_{2nd}) = \frac{\sum_{i=0}^{k} S_{y,i} W_{i,2nd}}{\sum_{i=0}^{k} W_{i,2nd}} \quad (3.38)$$

3.4. Design of the fuzzy set

To interpolate the concentration in the range of the boundary points, the membership function is the triangle membership function. In the ideal case, the number and the position of boundary points do not affect the result. However, in the case of the deviation of Beer-Lambert law, the number and the position of the boundary points affect the reduction of the error. This sub-section simulates the concentration calculation by the increase of boundary points and the movement of the position of boundary points. Figure 3.26 is the light absorbance which is the positive deviation of Beer-Lambert's law.

The 1st simulation is the increase of the boundary points without the movement of the boundary point position shown in figure 3.27. It shown that the calculated function is approximate to the function of the deviation of Beer-Lambert's law without receding from the deviation of Beer-Lambert's law in every light absorbance. Table 3.4 shows the average error of the concentration of solution calculated by proposed method of figure 3.27. When the number of the boundary points increase without the movement of the position, the average error of concentration reduces.

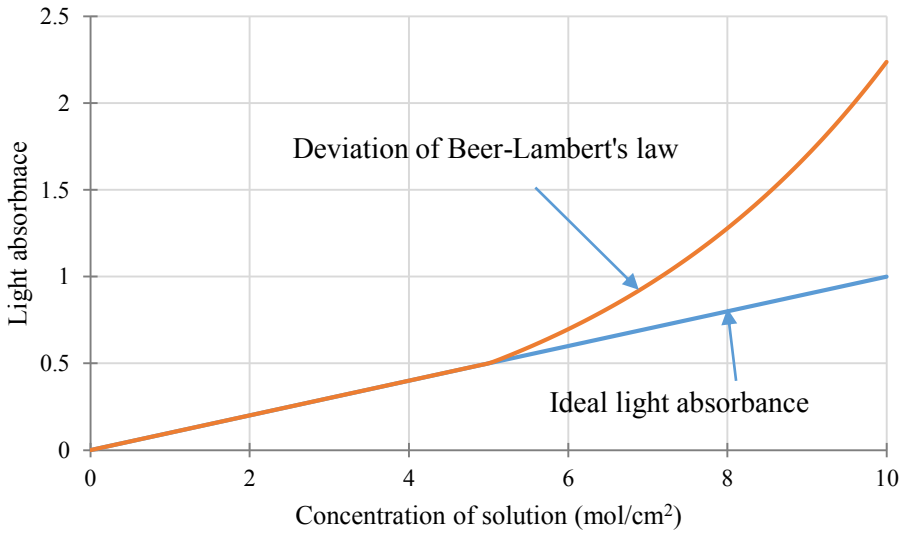


Figure 3.26. Example input light absorbance.

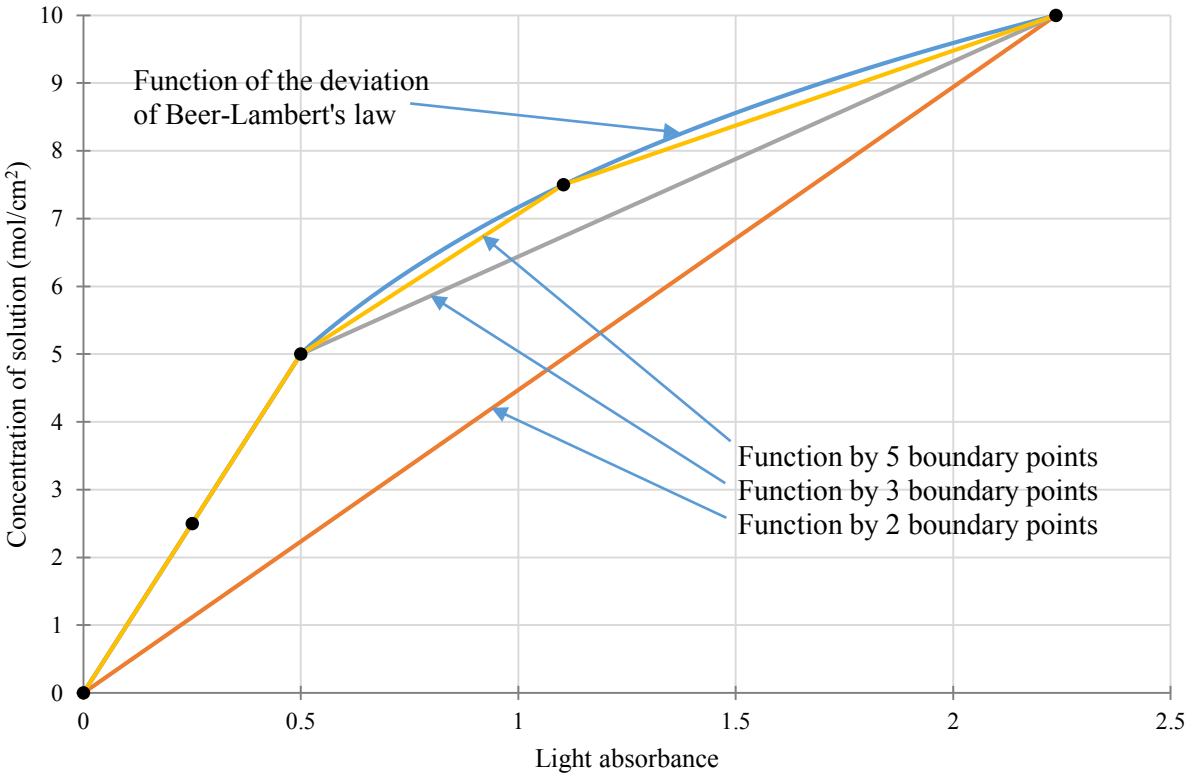


Figure 3.27. Simulation of the increase of the boundary points without movement of position.

Table 3.4. Average error of concentration (increase of boundary points).

Number of boundary points	Average error
2	1.761
3	0.253
5	0.065

Table 3.5. Position of the boundary points in the 2nd simulation.

	c_0	c_1	c_2	c_3
1st case	0	3.3	6.6	10
2nd case	0	5	7.5	10
3rd case	0	2.5	5	10

Table 3.6. Average error of concentration (change of the position of boundary points).

	Average Error
1st case	0.191
2nd case	0.065
3rd case	0.252

The 2nd simulation is the movement of the boundary point position shown in figure 3.28. The simulation has 3 cases. In 1st case, the boundary points are set by each range of boundary points is equal in direction of concentration. In 2nd case, many boundary points are set in the error area. In 3rd case, many boundary points are set at none error area. The position of the boundary points is shown in table 3.5. Table 3.6 shows that the average error of the concentration of 3 cases. It shows that the average error of 2nd case is the least. Therefore, the boundary points should be set in the error area. In 3rd case, the point that the error increases from none error is in the range of boundary points. The number of error in that range is more than other cases. Therefore, the boundary points should be set at the point is the differential of the error is changed from 0 to positive or negative.

However, in the experiment, the deviation area is not known. Therefore, the best method to reduce the error is the increase of number of the boundary points.

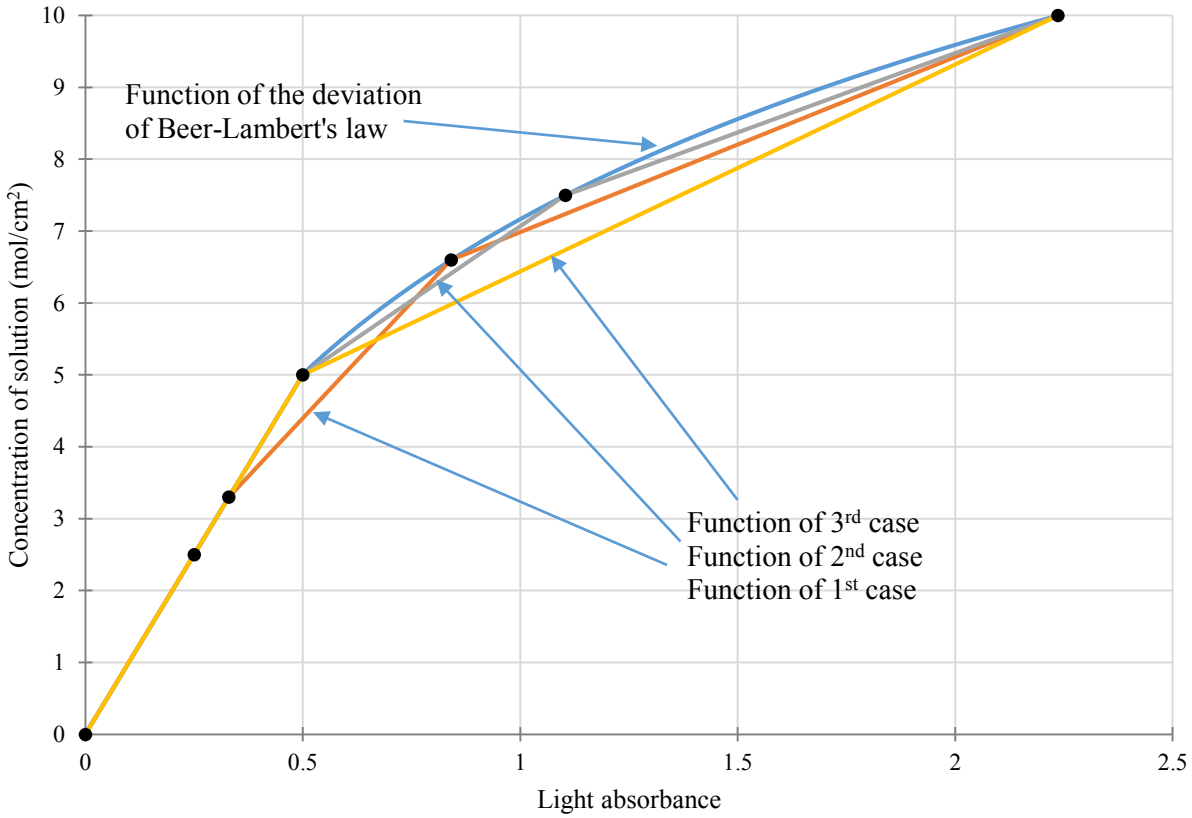


Figure 3.28. Simulation of the movement of the boundary point positions without change of the number of boundary points.

Table 4.1. Calculated concentration of the 1st component.

1st / 2nd	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2
0	0	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8	8.8	9.6	10.4	11.2	12	12.8	13.6	14.4	15.2	16
0.1	-0.4	0.4	1.2	2	2.8	3.6	4.4	5.2	6	6.8	7.6	8.4	9.2	10	10.8	11.6	12.4	13.2	14	14.8	15.6
0.2	-0.8	0	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8	8.8	9.6	10.4	11.2	12	12.8	13.6	14.4	15.2
0.3	-1.2	-0.4	0.4	1.2	2	2.8	3.6	4.4	5.2	6	6.8	7.6	8.4	9.2	10	10.8	11.6	12.4	13.2	14	14.8
0.4	-1.6	-0.8	0	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8	8.8	9.6	10.4	11.2	12	12.8	13.6	14.4
0.5	-2	-1.2	-0.4	0.4	1.2	2	2.8	3.6	4.4	5.2	6	6.8	7.6	8.4	9.2	10	10.8	11.6	12.4	13.2	14
0.6	-2.4	-1.6	-0.8	0	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8	8.8	9.6	10.4	11.2	12	12.8	13.6
0.7	-2.8	-2	-1.2	-0.4	0.4	1.2	2	2.8	3.6	4.4	5.2	6	6.8	7.6	8.4	9.2	10	10.8	11.6	12.4	13.2
0.8	-3.2	-2.4	-1.6	-0.8	0	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8	8.8	9.6	10.4	11.2	12	12.8
0.9	-3.6	-2.8	-2	-1.2	-0.4	0.4	1.2	2	2.8	3.6	4.4	5.2	6	6.8	7.6	8.4	9.2	10	10.8	11.6	12.4
1	-4	-3.2	-2.4	-1.6	-0.8	0	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8	8.8	9.6	10.4	11.2	12
1.1	-4.4	-3.6	-2.8	-2	-1.2	-0.4	0.4	1.2	2	2.8	3.6	4.4	5.2	6	6.8	7.6	8.4	9.2	10	10.8	11.6
1.2	-4.8	-4	-3.2	-2.4	-1.6	-0.8	0	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8	8.8	9.6	10.4	11.2
1.3	-5.2	-4.4	-3.6	-2.8	-2	-1.2	-0.4	0.4	1.2	2	2.8	3.6	4.4	5.2	6	6.8	7.6	8.4	9.2	10	10.8
1.4	-5.6	-4.8	-4	-3.2	-2.4	-1.6	-0.8	0	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8	8.8	9.6	10.4
1.5	-6	-5.2	-4.4	-3.6	-2.8	-2	-1.2	-0.4	0.4	1.2	2	2.8	3.6	4.4	5.2	6	6.8	7.6	8.4	9.2	10
1.6	-6.4	-5.6	-4.8	-4	-3.2	-2.4	-1.6	-0.8	0	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8	8.8	9.6
1.7	-6.8	-6	-5.2	-4.4	-3.6	-2.8	-2	-1.2	-0.4	0.4	1.2	2	2.8	3.6	4.4	5.2	6	6.8	7.6	8.4	9.2
1.8	-7.2	-6.4	-5.6	-4.8	-4	-3.2	-2.4	-1.6	-0.8	0	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8	8.8
1.9	-7.6	-6.8	-6	-5.2	-4.4	-3.6	-2.8	-2	-1.2	-0.4	0.4	1.2	2	2.8	3.6	4.4	5.2	6	6.8	7.6	8.4
2	-8	-7.2	-6.4	-5.6	-4.8	-4	-3.2	-2.4	-1.6	-0.8	0	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8

Table 4.2. Calculated concentration of the 2nd component.

1st / 2nd	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2
0	0	-0.6	-1.2	-1.8	-2.4	-3	-3.6	-4.2	-4.8	-5.4	-6	-6.6	-7.2	-7.8	-8.4	-9	-9.6	-10.2	-10.8	-11.4	-12
0.1	0.8	0.2	-0.4	-1	-1.6	-2.2	-2.8	-3.4	-4	-4.6	-5.2	-5.8	-6.4	-7	-7.6	-8.2	-8.8	-9.4	-10	-10.6	-11.2
0.2	1.6	1	0.4	-0.2	-0.8	-1.4	-2	-2.6	-3.2	-3.8	-4.4	-5	-5.6	-6.2	-6.8	-7.4	-8	-8.6	-9.2	-9.8	-10.4
0.3	2.4	1.8	1.2	0.6	0	-0.6	-1.2	-1.8	-2.4	-3	-3.6	-4.2	-4.8	-5.4	-6	-6.6	-7.2	-7.8	-8.4	-9	-9.6
0.4	3.2	2.6	2	1.4	0.8	0.2	-0.4	-1	-1.6	-2.2	-2.8	-3.4	-4	-4.6	-5.2	-5.8	-6.4	-7	-7.6	-8.2	-8.8
0.5	4	3.4	2.8	2.2	1.6	1	0.4	-0.2	-0.8	-1.4	-2	-2.6	-3.2	-3.8	-4.4	-5	-5.6	-6.2	-6.8	-7.4	-8
0.6	4.8	4.2	3.6	3	2.4	1.8	1.2	0.6	0	-0.6	-1.2	-1.8	-2.4	-3	-3.6	-4.2	-4.8	-5.4	-6	-6.6	-7.2
0.7	5.6	5	4.4	3.8	3.2	2.6	2	1.4	0.8	0.2	-0.4	-1	-1.6	-2.2	-2.8	-3.4	-4	-4.6	-5.2	-5.8	-6.4
0.8	6.4	5.8	5.2	4.6	4	3.4	2.8	2.2	1.6	1	0.4	-0.2	-0.8	-1.4	-2	-2.6	-3.2	-3.8	-4.4	-5	-5.6
0.9	7.2	6.6	6	5.4	4.8	4.2	3.6	3	2.4	1.8	1.2	0.6	0	-0.6	-1.2	-1.8	-2.4	-3	-3.6	-4.2	-4.8
1	8	7.4	6.8	6.2	5.6	5	4.4	3.8	3.2	2.6	2	1.4	0.8	0.2	-0.4	-1	-1.6	-2.2	-2.8	-3.4	-4
1.1	8.8	8.2	7.6	7	6.4	5.8	5.2	4.6	4	3.4	2.8	2.2	1.6	1	0.4	-0.2	-0.8	-1.4	-2	-2.6	-3.2
1.2	9.6	9	8.4	7.8	7.2	6.6	6	5.4	4.8	4.2	3.6	3	2.4	1.8	1.2	0.6	0	-0.6	-1.2	-1.8	-2.4
1.3	10.4	9.8	9.2	8.6	8	7.4	6.8	6.2	5.6	5	4.4	3.8	3.2	2.6	2	1.4	0.8	0.2	-0.4	-1	-1.6
1.4	11.2	10.6	10	9.4	8.8	8.2	7.6	7	6.4	5.8	5.2	4.6	4	3.4	2.8	2.2	1.6	1	0.4	-0.2	-0.8
1.5	12	11.4	10.8	10.2	9.6	9	8.4	7.8	7.2	6.6	6	5.4	4.8	4.2	3.6	3	2.4	1.8	1.2	0.6	0
1.6	12.8	12.2	11.6	11	10.4	9.8	9.2	8.6	8	7.4	6.8	6.2	5.6	5	4.4	3.8	3.2	2.6	2	1.4	0.8
1.7	13.6	13	12.4	11.8	11.2	10.6	10	9.4	8.8	8.2	7.6	7	6.4	5.8	5.2	4.6	4	3.4	2.8	2.2	1.6
1.8	14.4	13.8	13.2	12.6	12	11.4	10.8	10.2	9.6	9	8.4	7.8	7.2	6.6	6	5.4	4.8	4.2	3.6	3	2.4
1.9	15.2	14.6	14	13.4	12.8	12.2	11.6	11	10.4	9.8	9.2	8.6	8	7.4	6.8	6.2	5.6	5	4.4	3.8	3.2
2	16	15.4	14.8	14.2	13.6	13	12.4	11.8	11.2	10.6	10	9.4	8.8	8.2	7.6	7	6.4	5.8	5.2	4.6	4

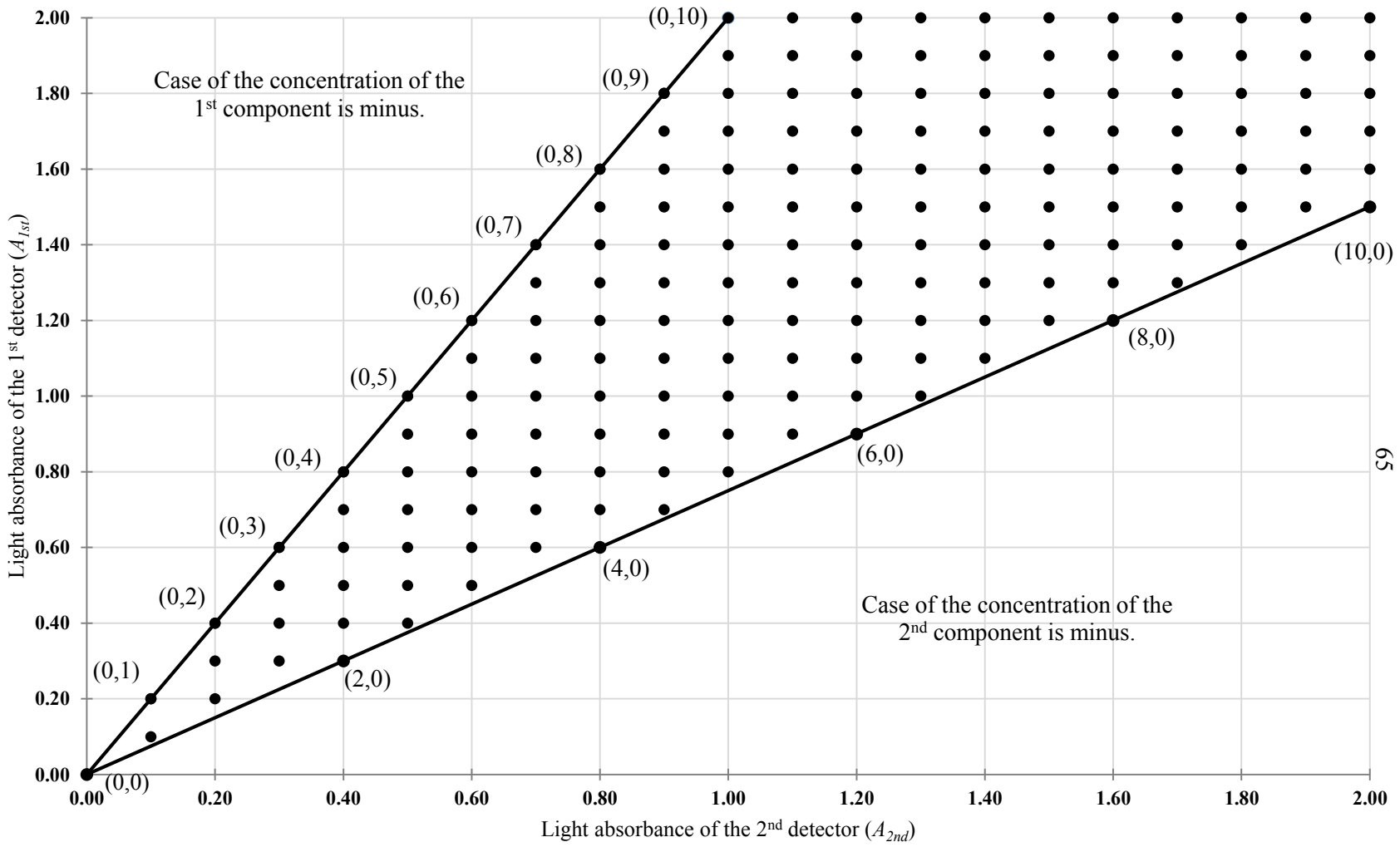


Figure 4.1. Possible concentration case which the concentration of the component is not minus (c_x, c_y) (contour graph).

4. Comparison of spectrophotometric method between the proposed method and the previous method

This section simulates the calculation of spectrophotometric method between the previous method and the proposed method. Then, the result of the proposed method is compared with the results of the previous method in the ideal case and the deviation of Beer-Lambert's law case. The compared previous methods are the simultaneous equation and the absorbance ratio method because these 2 methods can be used in all spectrophotometers and all cases of the wavelength and solution. In the ideal case, the ideal light absorbances of the 1st detector and the 2nd detector in table 3.1 and 3.2 are provided to calculate the concentration of solution. The concentrations of the components by every method are equal in the ideal case. The simulation result of the 1st component and the 2nd component are shown in table 4.1 and 4.2, respectively. The surface graph of the concentration of the component x and component y are shown in figure 3.15 and 3.16, respectively. The simulation result is calculated by the light absorbance from 0 to 2. Therefore, there is the impossible case of the solution which the concentration of the component is lesser than 0. Figure 4.1 exhibits the possible concentration case which the concentration of the component is not minus (c_x, c_y). The upper line is the concentration of the component x is 0. The below line is the concentration of the component y is 0. Therefore, the ideal case cannot be compared. We compare the result in the deviation of Beer-Lambert's law case.

4.1. Comparison in the case of the pure solution

In this subsection, the proposed method is compared with the concentration calculation by linear regression analysis in the case of the pure solution. The simulation provides the light absorbance of the deviation of Beer-Lambert's law in the figure 3.1.

Figure 4.2 shown the concentration function calculated by linear regression analysis. When the number of the known concentration data which are utilized to calculate the linear regression analysis increase, all concentrations are reduced to make the average concentration approximating to the ideal concentration. Table 4.3 shows the average error of concentration between the calculated concentration and the concentration of the deviation of Beer-Lambert's law (Ideal concentration). The average error is reduced when the number of the known concentration solution increases. However, when some concentration is approximate to the ideal concentration, some concentration is fairly different from the ideal concentration. Figure 4.3 displays the concentration function calculated by the proposed method. The number of the known concentration solution are same as the concentration function calculated by linear regression analysis. However, the concentration functions calculated by the proposed method are closely approximate to the ideal concentration which is function of the deviation of Beer-Lambert's law in every increase of the known concentration data.

Table 4.3. Average error of concentration of each method.

Number of the known concentration solutions	Linear regression	Proposed method
2	1.811	1.811
3	1.139	0.475
4	0.947	0.212
5	0.863	0.122
6	0.817	0.078

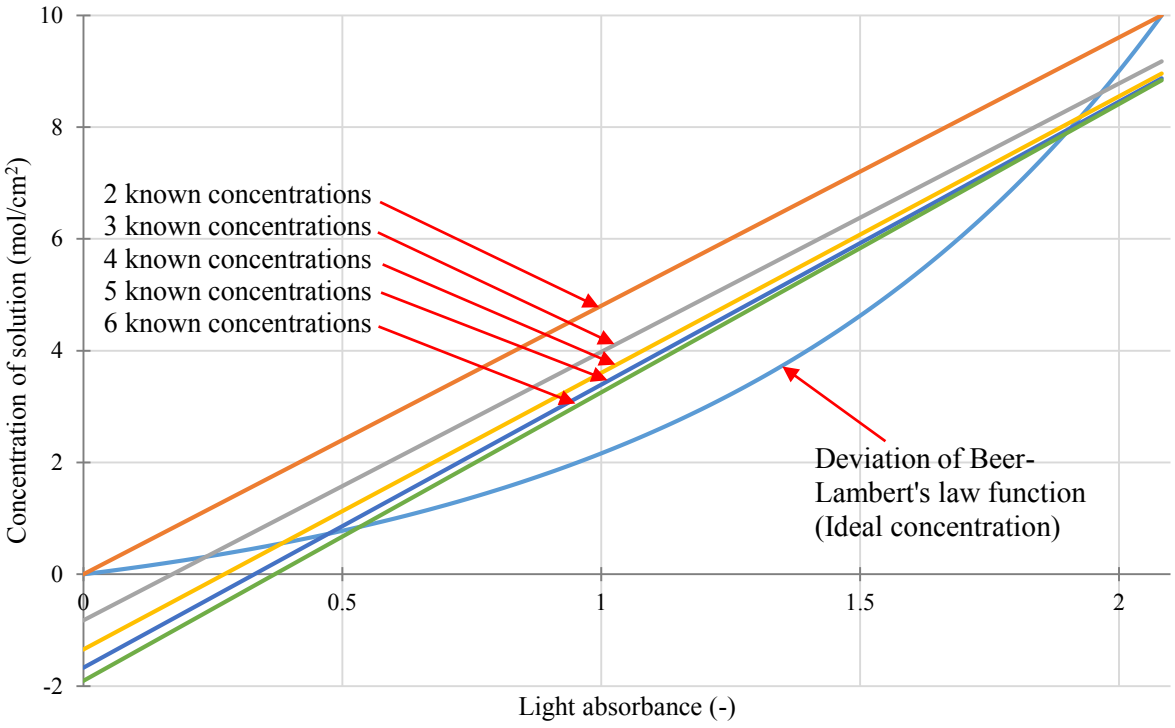


Figure 4.2. Function calculated by linear regression analysis.

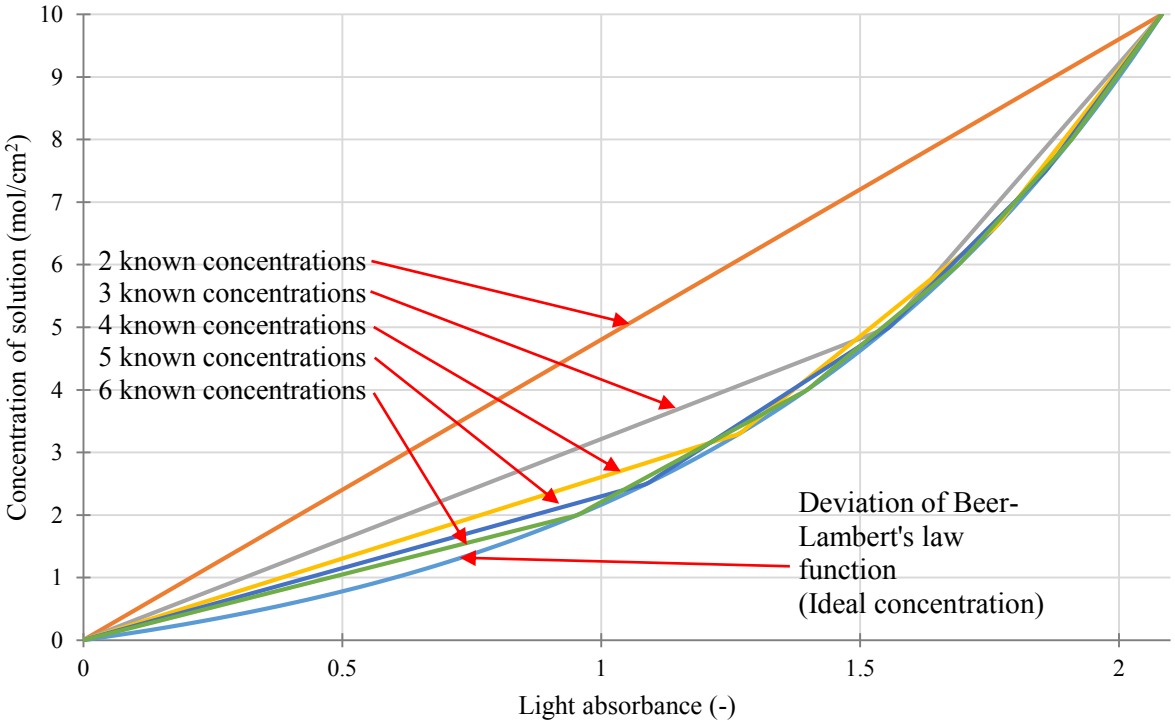


Figure 4.3. Function calculated by the proposed method.

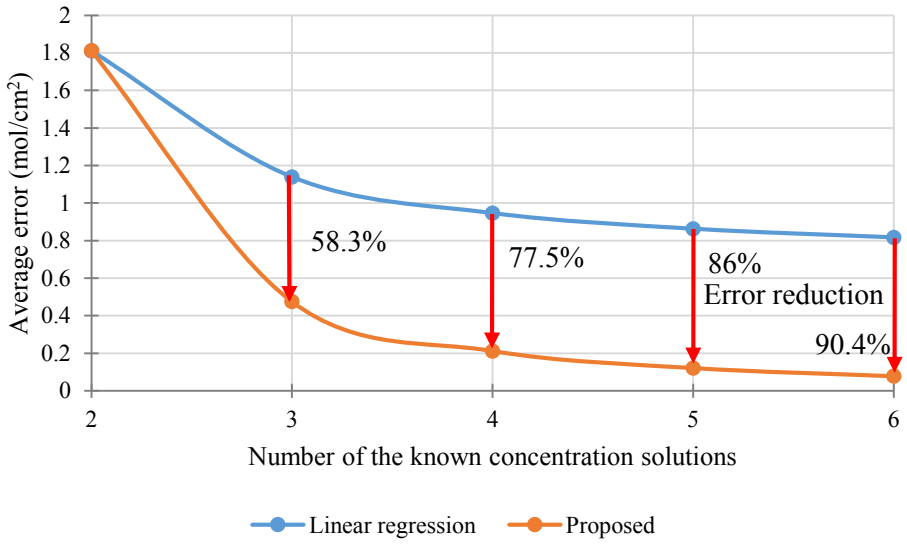


Figure 4.4. Comparison of average error by linear regression analysis and the proposed method.

Furthermore, the reduction of the average error by the proposed method is higher than the reduction of the average error shown in figure 4.4. In the case of 2 known concentration solutions, the average error of concentration between previous method and the proposed method are similar. However, when the number of the known concentration solution are more than 3, the average error of concentration by the proposed method is lesser than the average error of concentration by the previous method. The average error is reduced about 58.3% in the case of the 3 known concentration solutions by the proposed method. The reduction is higher to 77.5%, 86% and 90.4% when the number of the known concentration solutions increase to 4, 5 and 6, respectively. In addition, the concentration function calculated by the proposed method is approximate to the ideal concentration without receding from the ideal concentration in every light absorbance when the boundary points are increased without the movement of the position. This approximation is observed in the case of the 2, 3 and 5 boundary points in figure 4.3.

4.2. Comparison in the case of the multicomponent solution

In this sub-section, the calculation of the concentration of the component in the 2-component solution between the proposed method and the previous method is compared in the case of the deviation of Beer-Lambert's law. The simulation provides the light absorbance of the pure solution of component *x* and *y* in figure 4.5 and 4.6 which is the light absorbance of the 1st detector and the 2nd detector, respectively. The light absorbance of the 1st detector is the deviation of Beer-Lambert's law light absorbance. The light absorbance of the 2nd detector is the ideal light absorbance. The light absorbance of the mixture solution in and level concentration of component of the 1st detector and the 2nd detector are provided as input shown in table 4.4 and 4.5, respectively.

The calculated average concentrations of the component *x* and *y* by the simultaneous equation method are shown in figure 4.7 and 4.8, respectively. Although the number of the known concentration solutions increase, the calculated concentrations do not change much. The average data reduces only a little bit. The calculated concentration by the 2 and 3 known concentration solutions per one component is equal because the molar absorptivity calculated from the known concentration data by linear regression analysis is the same. The

calculated average concentrations of the component *x* and *y* by the absorbance ratio method are shown in figure 4.9 and 4.10, respectively. The calculated concentration is approximate to the calculated concentration by the simultaneous equation method because the standard solution provides the molar absorptivity. Although the number of the known concentration solution increase, the calculated concentrations do not change the same amount as to the simultaneous equation method. The average data reduces only a little bit. The calculated concentration by the 2 and 3 known concentration solution per one component is equal same as the simultaneous equation method. The calculated average concentrations of the component *x* and *y* by the proposed method are shown in figure 4.11 and 4.12, respectively. The calculated average concentrations by 2 known concentration solutions and the proposed method are the same as the calculated average concentrations by 2 known concentration solutions and the previous method. However, when the number of the known concentration solution increase, the concentrations are much more approximate to the ideal concentration.

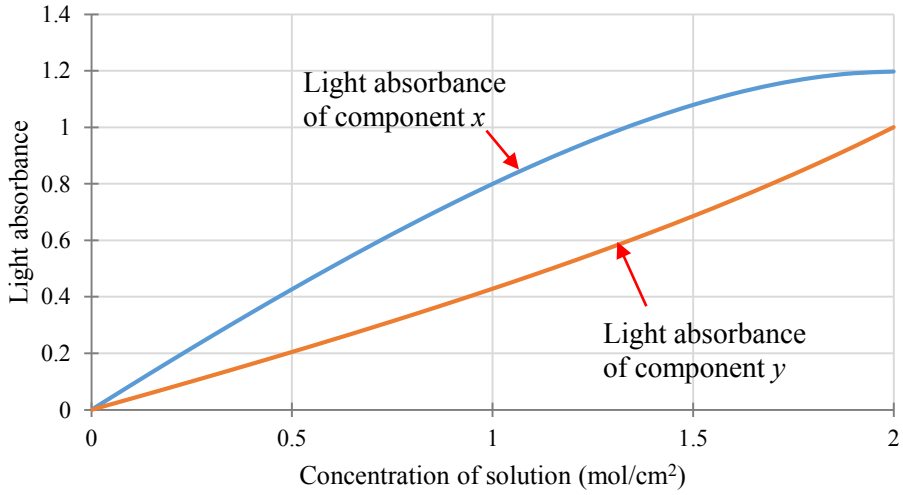


Figure 4.5. Light absorbance of the 1st detector.

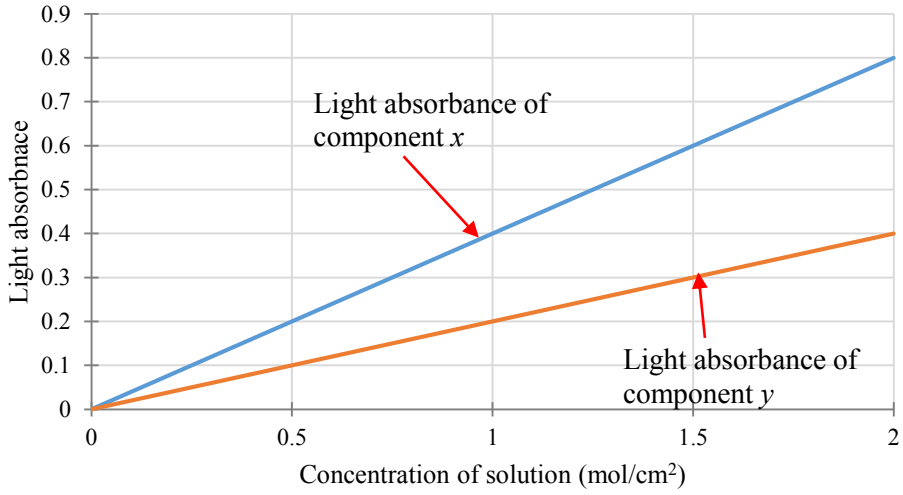


Figure 4.6. Light absorbance of the 2nd detector.

Table 4.4. Light absorbance of the 1st detector (A_{1st}).

c_x \ c_y	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1**	**1.1**	**1.2**	**1.3**	**1.4**	**1.5**	**1.6**	**1.7**	**1.8**	**1.9**	**2**
0	0.000	0.040	0.080	0.121	0.163	0.205	0.248	0.291	0.336	0.382	0.429	0.477	0.527	0.578	0.631	0.686	0.743	0.803	0.866	0.932	1.001
0.1	0.088	0.128	0.169	0.209	0.251	0.293	0.336	0.380	0.424	0.470	0.517	0.565	0.615	0.666	0.719	0.774	0.832	0.892	0.954	1.020	1.089
0.2	0.175	0.215	0.256	0.296	0.338	0.380	0.423	0.467	0.511	0.557	0.604	0.652	0.702	0.753	0.806	0.861	0.919	0.979	1.041	1.107	1.176
0.3	0.261	0.301	0.341	0.382	0.423	0.466	0.509	0.552	0.597	0.643	0.690	0.738	0.787	0.839	0.892	0.947	1.004	1.064	1.127	1.193	1.262
0.4	0.345	0.385	0.425	0.466	0.507	0.549	0.592	0.636	0.681	0.727	0.773	0.822	0.871	0.923	0.976	1.031	1.088	1.148	1.211	1.276	1.346
0.5	0.427	0.467	0.507	0.548	0.589	0.631	0.674	0.718	0.763	0.809	0.855	0.904	0.953	1.004	1.058	1.113	1.170	1.230	1.292	1.358	1.428
0.6	0.507	0.546	0.587	0.628	0.669	0.711	0.754	0.798	0.843	0.888	0.935	0.983	1.033	1.084	1.137	1.192	1.250	1.310	1.372	1.438	1.507
0.7	0.584	0.624	0.664	0.705	0.747	0.789	0.832	0.875	0.920	0.966	1.013	1.061	1.111	1.162	1.215	1.270	1.327	1.387	1.450	1.516	1.585
0.8	0.659	0.699	0.739	0.780	0.822	0.864	0.907	0.950	0.995	1.041	1.088	1.136	1.185	1.237	1.290	1.345	1.402	1.462	1.525	1.591	1.660
0.9	0.731	0.771	0.811	0.852	0.893	0.936	0.979	1.022	1.067	1.113	1.160	1.208	1.257	1.309	1.362	1.417	1.474	1.534	1.597	1.662	1.732
1	0.800	0.839	0.880	0.921	0.962	1.004	1.047	1.091	1.136	1.181	1.228	1.276	1.326	1.377	1.430	1.486	1.543	1.603	1.665	1.731	1.800
1.1	0.865	0.905	0.945	0.986	1.027	1.069	1.112	1.156	1.201	1.246	1.293	1.342	1.391	1.442	1.496	1.551	1.608	1.668	1.730	1.796	1.866
1.2	0.926	0.966	1.006	1.047	1.088	1.130	1.173	1.217	1.262	1.307	1.354	1.403	1.452	1.503	1.557	1.612	1.669	1.729	1.791	1.857	1.927
1.3	0.982	1.022	1.062	1.103	1.145	1.187	1.230	1.274	1.318	1.364	1.411	1.459	1.509	1.560	1.613	1.668	1.725	1.785	1.848	1.914	1.983
1.4	1.034	1.073	1.114	1.155	1.196	1.238	1.281	1.325	1.370	1.415	1.462	1.510	1.560	1.611	1.664	1.720	1.777	1.837	1.899	1.965	2.034
1.5	1.079	1.119	1.160	1.200	1.242	1.284	1.327	1.371	1.415	1.461	1.508	1.556	1.606	1.657	1.710	1.765	1.823	1.882	1.945	2.011	2.080
1.6	1.119	1.159	1.199	1.240	1.281	1.324	1.366	1.410	1.455	1.501	1.547	1.596	1.645	1.697	1.750	1.805	1.862	1.922	1.985	2.050	2.120
1.7	1.151	1.191	1.231	1.272	1.314	1.356	1.399	1.443	1.487	1.533	1.580	1.628	1.678	1.729	1.782	1.837	1.894	1.954	2.017	2.083	2.152
1.8	1.176	1.216	1.256	1.297	1.338	1.380	1.423	1.467	1.512	1.558	1.604	1.653	1.702	1.754	1.807	1.862	1.919	1.979	2.042	2.107	2.177
1.9	1.192	1.231	1.272	1.313	1.354	1.396	1.439	1.483	1.528	1.573	1.620	1.668	1.718	1.769	1.822	1.877	1.935	1.995	2.057	2.123	2.192
2	1.197	1.237	1.278	1.319	1.360	1.402	1.445	1.489	1.534	1.579	1.626	1.674	1.724	1.775	1.828	1.883	1.941	2.001	2.063	2.129	2.198

Table 4.5. Light absorbance of the 2nd detector (A_{2nd}).

c_x \ c_y	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1**	**1.1**	**1.2**	**1.3**	**1.4**	**1.5**	**1.6**	**1.7**	**1.8**	**1.9**	**2**
0	0	0.02	0.04	0.06	0.08	0.1	0.12	0.14	0.16	0.18	0.2	0.22	0.24	0.26	0.28	0.3	0.32	0.34	0.36	0.38	0.4
0.1	0.04	0.06	0.08	0.1	0.12	0.14	0.16	0.18	0.2	0.22	0.24	0.26	0.28	0.3	0.32	0.34	0.36	0.38	0.4	0.42	0.44
0.2	0.08	0.1	0.12	0.14	0.16	0.18	0.2	0.22	0.24	0.26	0.28	0.3	0.32	0.34	0.36	0.38	0.4	0.42	0.44	0.46	0.48
0.3	0.12	0.14	0.16	0.18	0.2	0.22	0.24	0.26	0.28	0.3	0.32	0.34	0.36	0.38	0.4	0.42	0.44	0.46	0.48	0.5	0.52
0.4	0.16	0.18	0.2	0.22	0.24	0.26	0.28	0.3	0.32	0.34	0.36	0.38	0.4	0.42	0.44	0.46	0.48	0.5	0.52	0.54	0.56
0.5	0.2	0.22	0.24	0.26	0.28	0.3	0.32	0.34	0.36	0.38	0.4	0.42	0.44	0.46	0.48	0.5	0.52	0.54	0.56	0.58	0.6
0.6	0.24	0.26	0.28	0.3	0.32	0.34	0.36	0.38	0.4	0.42	0.44	0.46	0.48	0.5	0.52	0.54	0.56	0.58	0.6	0.62	0.64
0.7	0.28	0.3	0.32	0.34	0.36	0.38	0.4	0.42	0.44	0.46	0.48	0.5	0.52	0.54	0.56	0.58	0.6	0.62	0.64	0.66	0.68
0.8	0.32	0.34	0.36	0.38	0.4	0.42	0.44	0.46	0.48	0.5	0.52	0.54	0.56	0.58	0.6	0.62	0.64	0.66	0.68	0.7	0.72
0.9	0.36	0.38	0.4	0.42	0.44	0.46	0.48	0.5	0.52	0.54	0.56	0.58	0.6	0.62	0.64	0.66	0.68	0.7	0.72	0.74	0.76
1	0.4	0.42	0.44	0.46	0.48	0.5	0.52	0.54	0.56	0.58	0.6	0.62	0.64	0.66	0.68	0.7	0.72	0.74	0.76	0.78	0.8
1.1	0.44	0.46	0.48	0.5	0.52	0.54	0.56	0.58	0.6	0.62	0.64	0.66	0.68	0.7	0.72	0.74	0.76	0.78	0.8	0.82	0.84
1.2	0.48	0.5	0.52	0.54	0.56	0.58	0.6	0.62	0.64	0.66	0.68	0.7	0.72	0.74	0.76	0.78	0.8	0.82	0.84	0.86	0.88
1.3	0.52	0.54	0.56	0.58	0.6	0.62	0.64	0.66	0.68	0.7	0.72	0.74	0.76	0.78	0.8	0.82	0.84	0.86	0.88	0.9	0.92
1.4	0.56	0.58	0.6	0.62	0.64	0.66	0.68	0.7	0.72	0.74	0.76	0.78	0.8	0.82	0.84	0.86	0.88	0.9	0.92	0.94	0.96
1.5	0.6	0.62	0.64	0.66	0.68	0.7	0.72	0.74	0.76	0.78	0.8	0.82	0.84	0.86	0.88	0.9	0.92	0.94	0.96	0.98	1
1.6	0.64	0.66	0.68	0.7	0.72	0.74	0.76	0.78	0.8	0.82	0.84	0.86	0.88	0.9	0.92	0.94	0.96	0.98	1	1.02	1.04
1.7	0.68	0.7	0.72	0.74	0.76	0.78	0.8	0.82	0.84	0.86	0.88	0.9	0.92	0.94	0.96	0.98	1	1.02	1.04	1.06	1.08
1.8	0.72	0.74	0.76	0.78	0.8	0.82	0.84	0.86	0.88	0.9	0.92	0.94	0.96	0.98	1	1.02	1.04	1.06	1.08	1.1	1.12
1.9	0.76	0.78	0.8	0.82	0.84	0.86	0.88	0.9	0.92	0.94	0.96	0.98	1	1.02	1.04	1.06	1.08	1.1	1.12	1.14	1.16
2	0.8	0.82	0.84	0.86	0.88	0.9	0.92	0.94	0.96	0.98	1	1.02	1.04	1.06	1.08	1.1	1.12	1.14	1.16	1.18	1.2

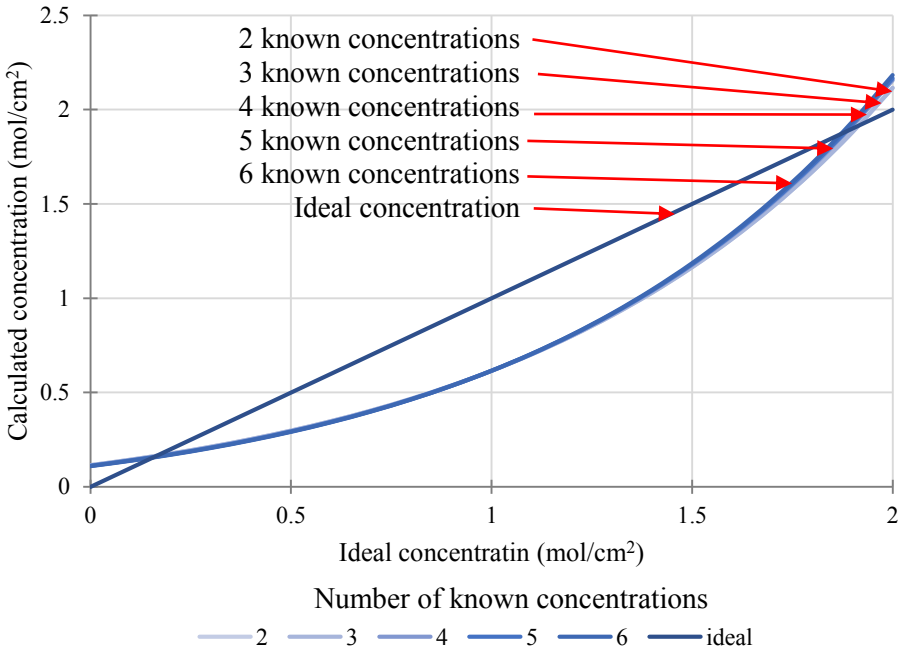


Figure 4.7. Calculated concentration of the component *x* by the simultaneous equation method.

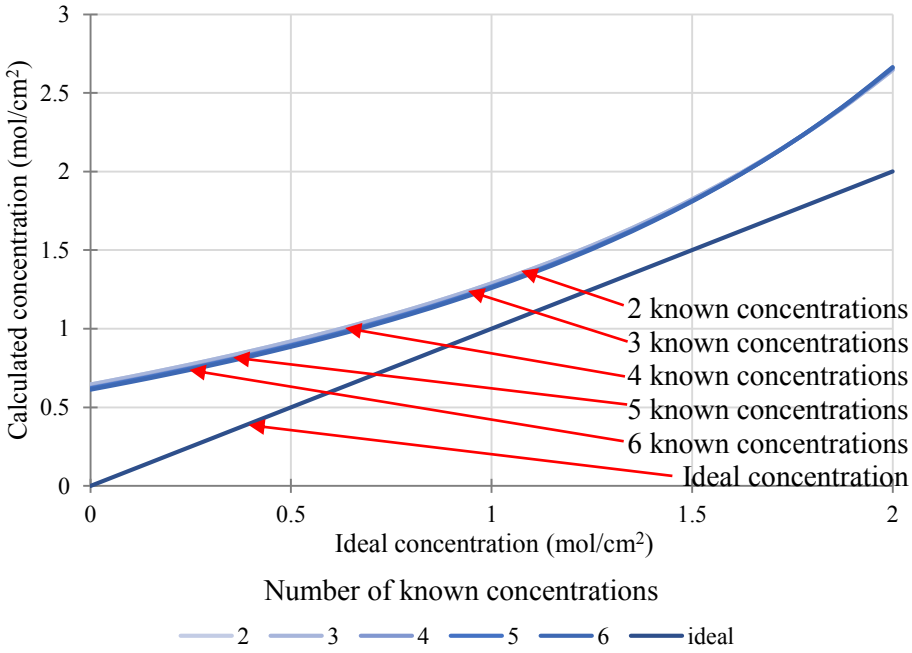


Figure 4.8. Calculated concentration of the component *y* by the simultaneous equation method.

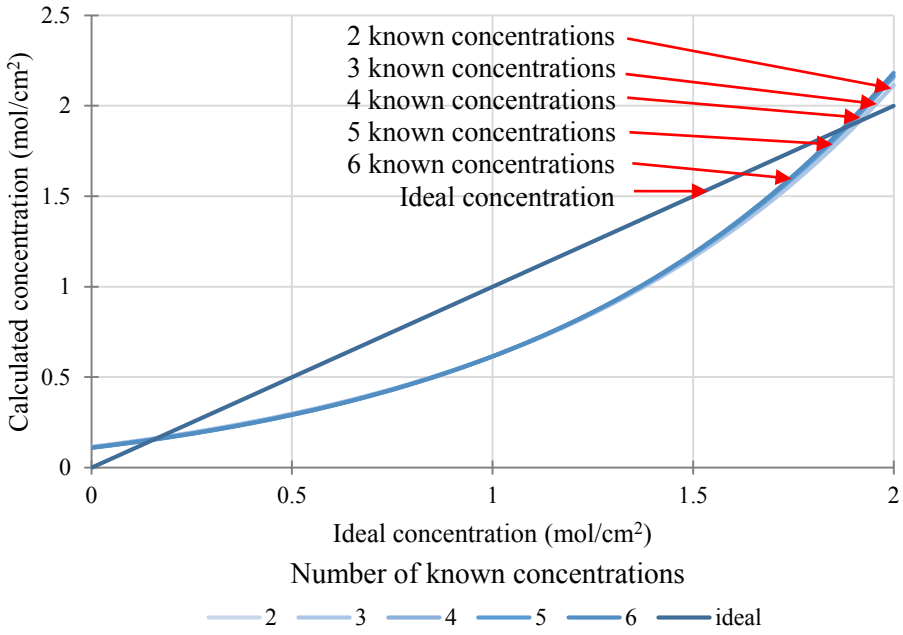


Figure 4.9. Calculated concentration of the component *x* by the absorbance ratio method.

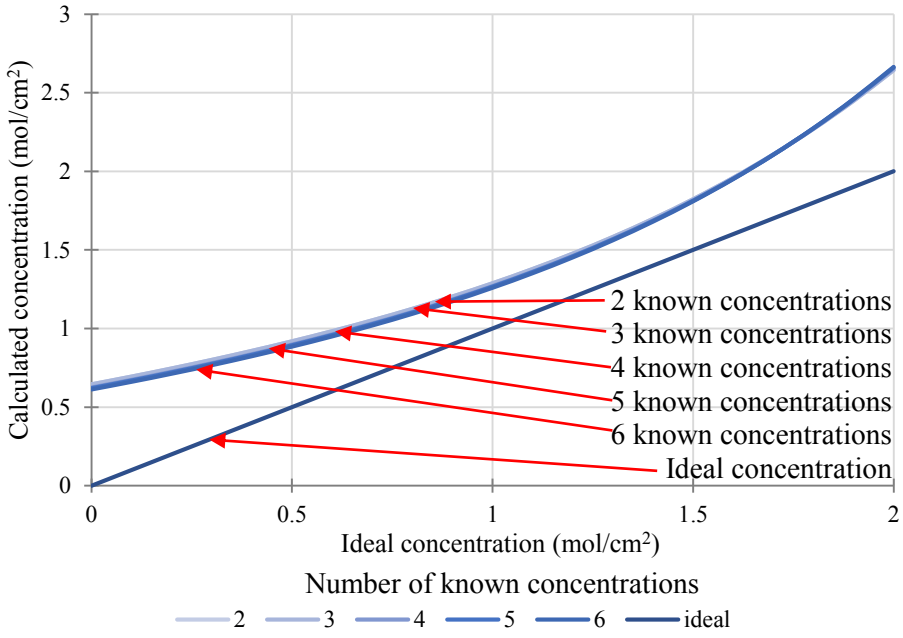


Figure 4.10. Calculated concentration of the component *y* by the absorbance ratio method.

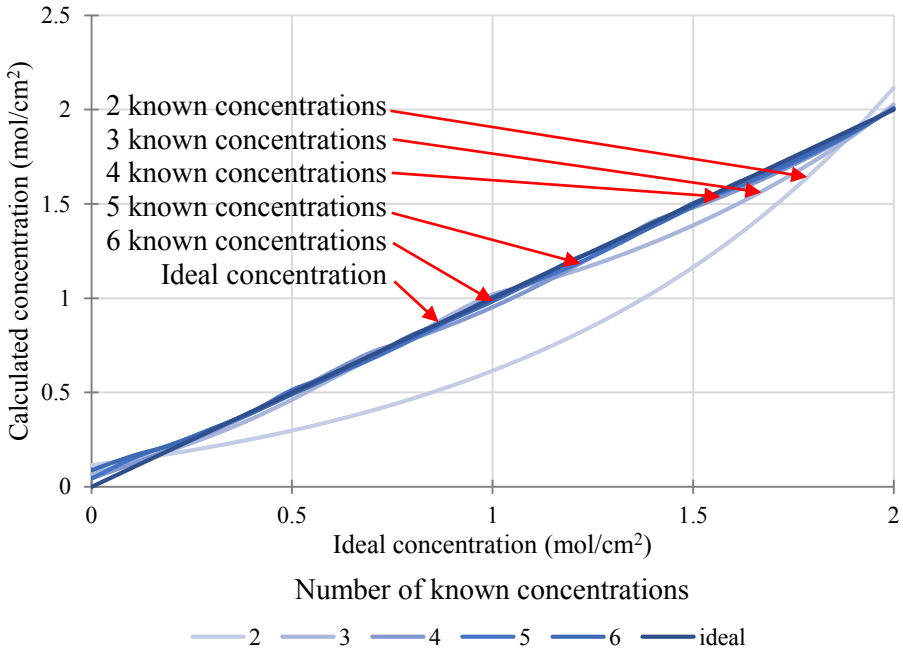


Figure 4.11. Calculated concentration of the component *x* by the proposed method.

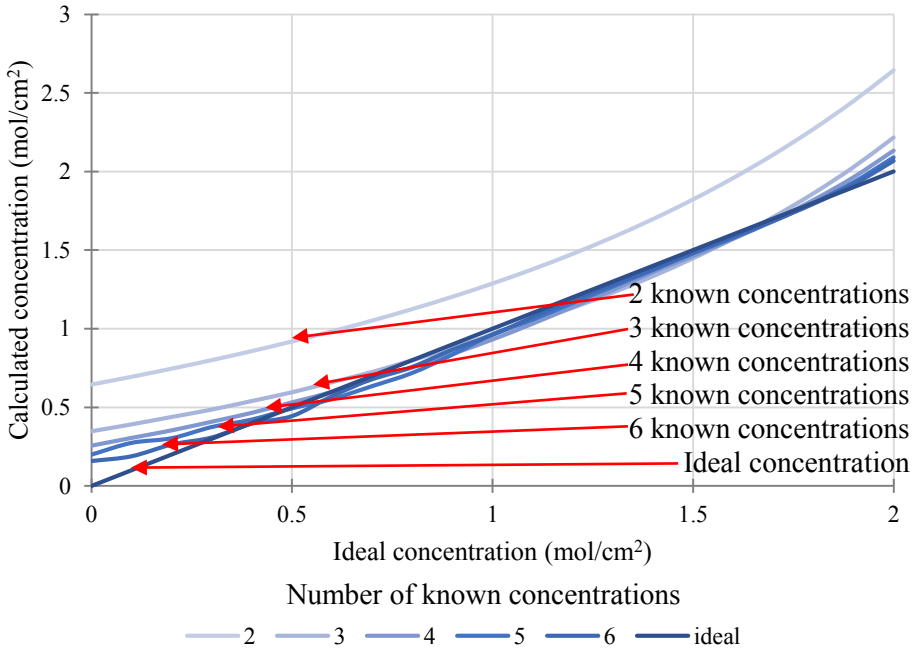


Figure 4.12. Calculated concentration of the component *y* by the proposed method.

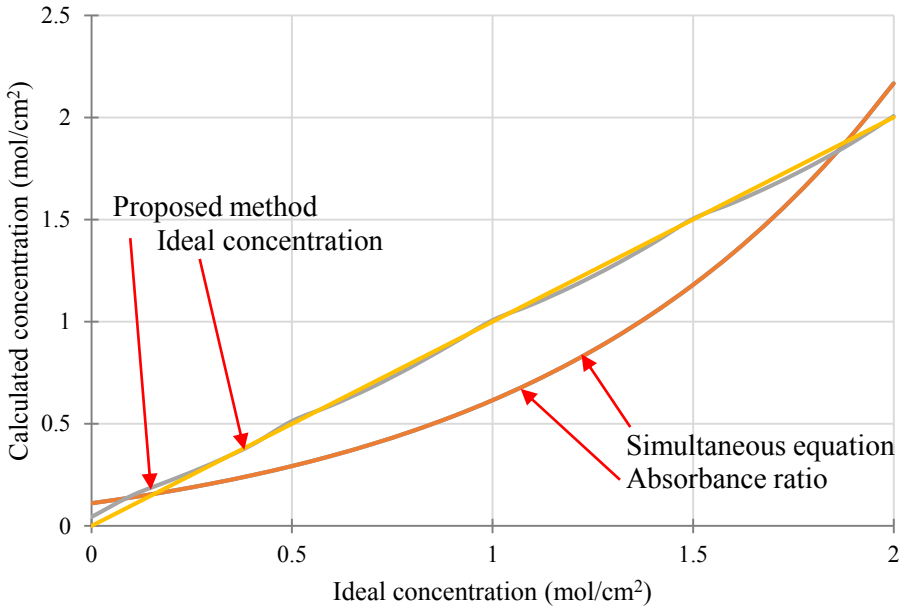


Figure 4.13. Calculated concentration of component *x* by 5 known concentration data.

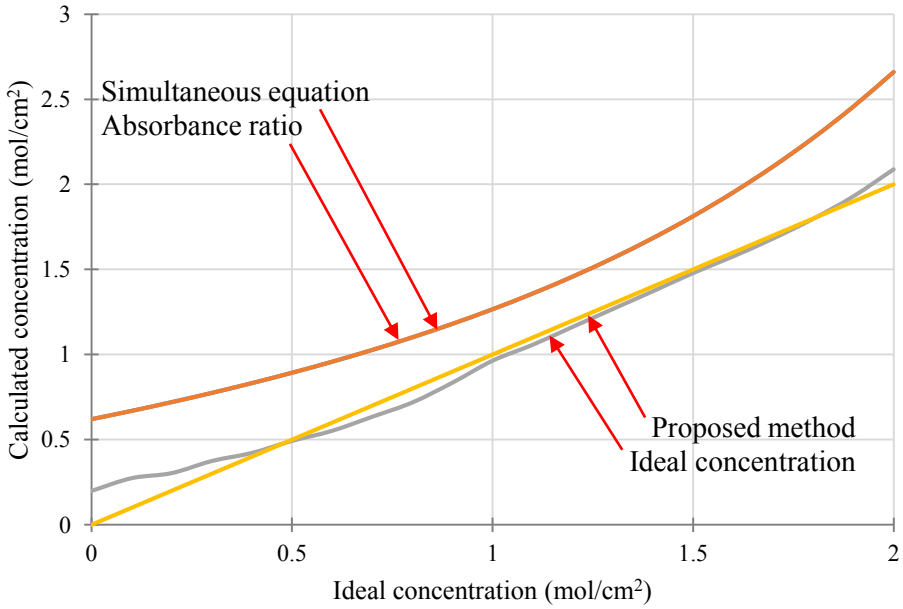


Figure 4.14. Calculated concentration of component *y* by 5 known concentration data.

Figure 4.13 and 4.14 displays the calculated concentrations by 5 known concentration data of component *x* and *y*, respectively. It shows that the calculated concentration by the proposed method is more approximate to the ideal concentration than the calculated concentration by the previous method.

Table 4.6 and figure 4.15 show the errors between the calculated concentration and the ideal concentration of component *x* when the number of the known concentration solution increases. Table 4.7 and figure 4.16 show the errors between the calculated concentration and the ideal concentration of component *y* when the number of the known concentration solution increase. When the number of the known concentration solution increase, the errors of average concentration are reduced. The error reduction of the previous method is lesser than the error reduction of the proposed method.

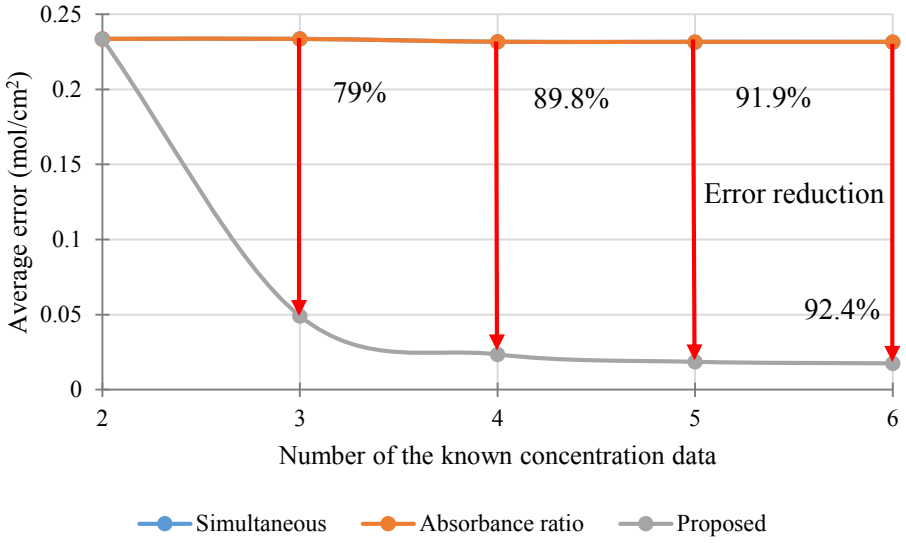


Figure 4.15. Average error of the concentration of the component *x*.

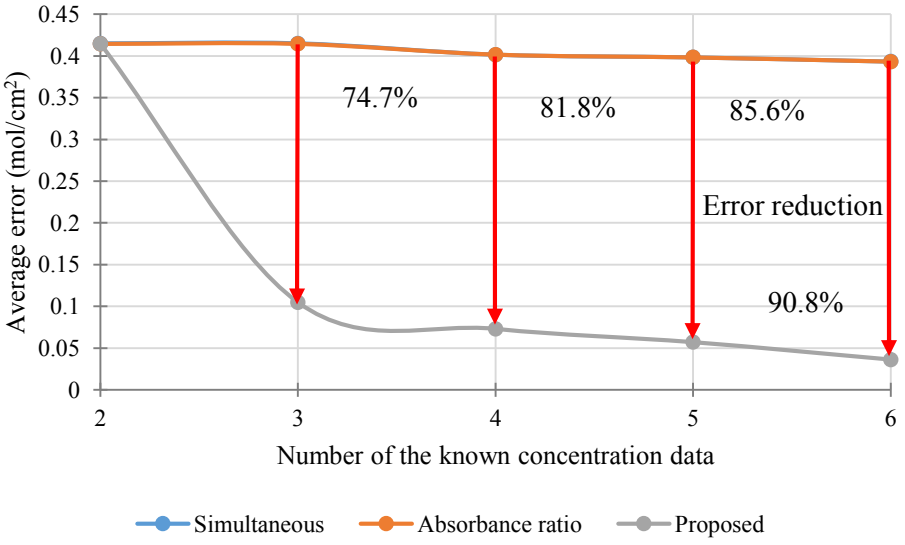


Figure 4.16. Average error of the concentration of the component *y*.

Table 4.6. Average error of the concentration of the component *x*.

Number of the known concentration solutions	Simultaneous method	Absorbance ratio method	Proposed method
2	0.234	0.234	0.233
3	0.234	0.234	0.049
4	0.232	0.232	0.024
5	0.232	0.232	0.019
6	0.232	0.232	0.018

Table 4.7. Average error of the concentration of the component *y*.

Number of the known concentration solutions	Simultaneous method	Absorbance ratio method	Proposed method
2	0.415	0.415	0.415
3	0.415	0.415	0.105
4	0.401	0.402	0.073
5	0.398	0.398	0.057
6	0.393	0.393	0.036

From the average error of the concentration in component *x* in table 4.6, when the number of the concentration is 2, the average errors of 3 methods are similar. However, when the number of known concentration solution increases to 3, 4 and more, the average data of the proposed is reduced about 79%, 89.8%, 91.9%, respectively while the average error of the previous method is reduced a slightly. In the case of the component *y,* it is the same as the case of the component *x* that when the number of the concentration is 2, the average errors of 3 methods are similar. When the number of known concentration solution increases to 3, 4 and more, the average data of the proposed is reduced about 74.7%, 81.8%, 85.6%, respectively while the average error of the previous method is reduced a little bit.

4.3. Experiment

To confirm the calculated concentration by the proposed device approximately to the ideal concentration than the previous method, the experiment is performed.

4.3.1. Experimental setup

There is the pre-process in the proposed method. The experiment provides the spectrophotometer of WPA CO7500 colorimeter as the experimental device. The known concentration light absorbance is required by the spectrophotometric method as the boundary points and to calculate the molar absorptivity in previous methods. Therefore, the preparation must make many solutions in any concentration level of the components as the known concentration solution. The solutions are prepared by food colorings. Each solution is made at one level as the original solution. The volume of one solution is 3.3 ml. The one concentration level of the component is 3.3 ml from the original solution and the remained volume is the distilled water.

For an example:

In the case of the mixed solution which consists of the level 3 of the 1st solution and the level 2 of the 2nd solution, the mixture solution consists of 0.9 ml of the 1st solution, 0.6 ml of the 2nd solution and the 1.8 ml distilled water.

Table 4.8. Known concentration light absorbance of 470 nm of wavelength.

	G0	G1	G2	G3	G4	G5
R0	0	0.34	0.59	0.77	0.98	1.11
R1	0.18	0.52	0.78	1.05	1.15	1.37
R2	0.37	0.77	0.98	1.18	1.45	1.57
R3	0.54	0.98	1.24	1.48	1.65	1.78
R4	0.75	1.12	1.5	1.7	1.92	2
R5	0.86	1.29	1.68	1.89	2	2

Table 4.9. Known concentration light absorbance of 490 nm of the wavelength.

	G0	G1	G2	G3	G4	G5
R0	0	0.13	0.24	0.32	0.43	0.49
R1	0.24	0.37	0.48	0.59	0.63	0.75
R2	0.51	0.65	0.71	0.78	0.93	1
R3	0.75	0.9	1.01	1.11	1.18	1.24
R4	1.05	1.1	1.3	1.35	1.45	1.53
R5	1.22	1.33	1.54	1.58	1.7	1.78

Table 4.10. Known concentration light absorbance of 580 nm of the wavelength.

	B0	B1	B2	B3	B4	B5
R0	0	0.1	0.19	0.26	0.34	0.39
R1	0.12	0.25	0.33	0.43	0.54	0.61
R2	0.23	0.32	0.43	0.52	0.62	0.69
R3	0.31	0.44	0.49	0.64	0.7	0.77
R4	0.4	0.51	0.56	0.67	0.73	0.86
R5	0.48	0.55	0.6	0.68	0.85	0.9

Table 4.11. Known concentration light absorbance of 550 nm of the wavelength.

	B0	B1	B2	B3	B4	B5
R0	0	0.06	0.1	0.14	0.17	0.2
R1	0.45	0.51	0.63	0.72	0.77	0.82
R2	0.85	0.86	0.99	1.03	1.13	1.17
R3	1.13	1.27	1.27	1.33	1.43	1.49
R4	1.35	1.48	1.45	1.55	1.5	1.68
R5	1.49	1.56	1.61	1.63	1.8	1.81

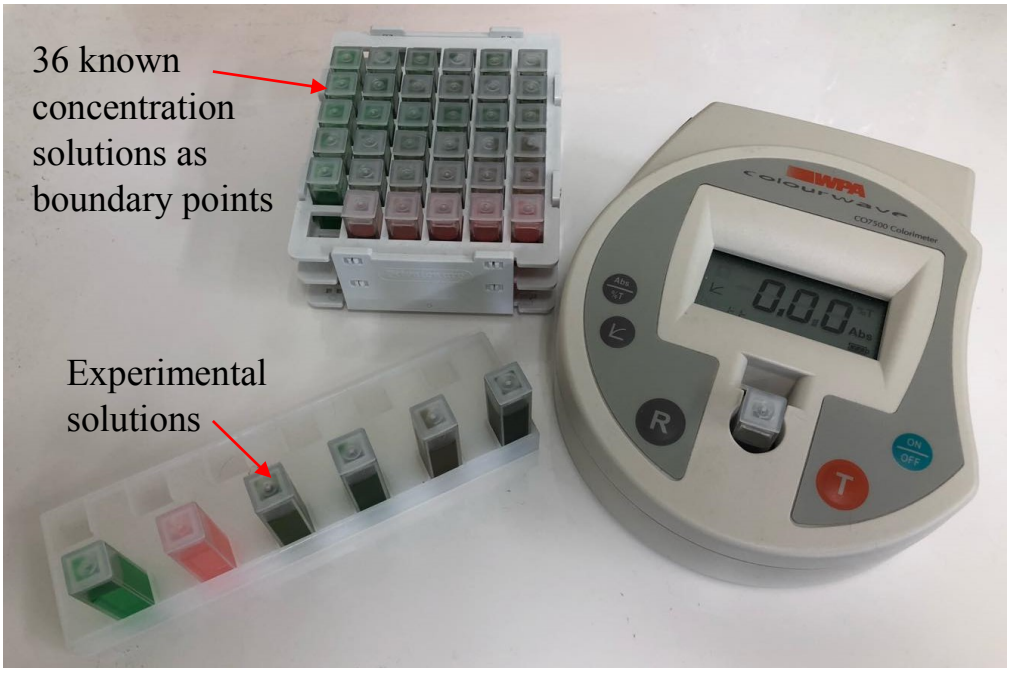


Figure 4.17. 1st experiment of the mixture solution between red component and green component.

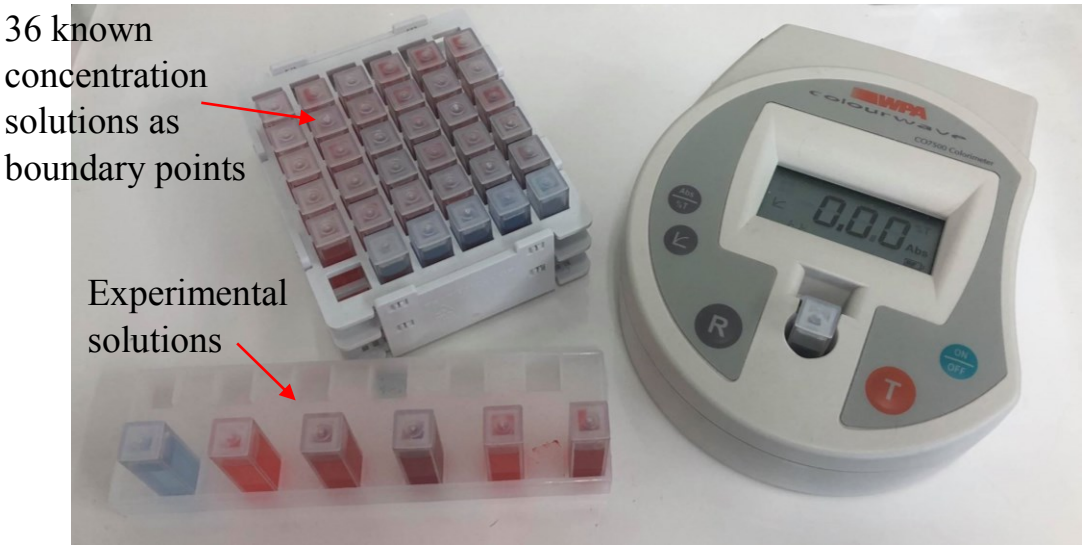


Figure 4.18. 2nd experiment of the mixture solution between red component and blue component.

After preparing the solution, they are measured by the 2 wavelengths of a light source. The light absorbance and the concentration of known concentration solution are utilized as the boundary points.

There are 2 experiments. The 1st experiment of the measurement of the mixture solution between the red solution and green solution shown in figure 4.17. The known concentration light absorbance of the 1st experiment is exhibited in table 4.8 and 4.9 which is measured by 470nm and 490nm of the wavelengths. The

2nd experiment is the measurement of the mixture solution between the red solution and blue solution shown in figure 4.18. The known concentration light absorbance of the 2nd experiment is exhibited in table 4.10 and 4.11 which is measured by 580nm and 550nm of the wavelengths. The mixture solutions in any concentration level of each component are measured by many methods in experiment. After that, the result is provided to calculate the concentration error between the ideal and any calculated methods.

4.3.2. Experimental result

The 1st experiment utilizes the light absorbance of the 470nm wavelength as the 1st light absorbance and the red solution as the 1st component. The result of the 1st experiment is shown in table 4.12. There are the concentrations of the component which are calculated by the proposed method, simultaneous equation method and the absorbance ratio method. The 2nd experiment provides the light absorbance of the 580nm wavelength as the 1st light absorbance and the red solution as the 1st component. The result of the 2nd experiment is shown in table 4.13. There is the concentration of the component which is calculated by the proposed method, simultaneous equation method and the absorbance ratio method. The calculated concentration result of the simultaneous equation and absorbance ratio are similar because the light absorbance of the standard solution provides the molar absorptivity.

Table 4.14 and 4.15 shows the different between the ideal concentration and the calculated concentration by the any spectrophotometric of 1st experiment and 2nd experiment, respectively. The average different between the ideal concentration and the concentration calculated by the proposed method is lesser than the average different between the ideal concentration and the concentration calculated by the previous method. It shows that the proposed method can reduce the error from the proposed method. As the different result, the reduction of the error by the proposed method of 1st experiment and 2nd experiment can be calculated shown in table 4.16 and 4.17, respectively. From the average error reduction result and the average error result, the percentage error reduction of the 1st experiment and 2nd experiment are shown in table 4.18 and 4.19, respectively. In the 1st experiment, the proposed method can reduce the error from the previous method about 69.437% in the case of the red component and 93.386% in the case of the green component. In the 2nd experiment, the proposed method can reduce the error from the previous method about 83.7% in the case of the red component and 55.85% in the case of the blue component.

Table 4.12. Concentration of the component calculated by any method of the 1st experiment.

Volume		**Ideal Concentration**		**Light absorbance**		**Proposed**		**Simultaneous equation**		**Absorbance ratio**	
Red	**Green**	**Red**	**Green**	**470nm**	**490nm**	**Red**	**Green**	**Red**	**Green**	**Red**	**Green**
0	5	0	1.66	0.47	0.18	-0.043	1.548	-0.179	2.295	-0.179	2.295
5	0	1.66	0	0.31	0.43	1.717	-0.071	1.698	0.047	1.698	0.047
5	5	1.66	1.66	0.85	0.63	1.739	1.610	1.452	2.715	1.452	2.716
5	10	1.66	3.33	1.25	0.8	1.859	3.427	1.398	4.589	1.397	4.59
10	5	3.33	1.66	1.2	1.05	3.382	1.603	2.987	3.077	2.987	3.077
10	10	3.33	3.33	1.61	1.22	3.344	3.346	2.906	5.017	2.906	5.018

Table 4.13. Concentration of the component calculated by any method of the 2nd experiment.

Volume		Ideal Concentration		Light absorbance		Proposed		Simultaneous equation		Absorbance ratio	
Red	**Blue**	**Red**	**Blue**	**580nm**	**550nm**	**Red**	**Blue**	**Red**	**Blue**	**Red**	**Blue**
0	5	0	1.66	0.47	0.18	-0.072	1.745	-0.039	2.091	-0.039	2.092
5	0	1.66	0	0.31	0.43	1.857	0.121	2.734	-0.376	2.734	-0.376
5	5	1.66	1.66	0.85	0.63	1.830	1.893	2.834	1.674	2.834	1.674
5	10	1.66	3.33	1.25	0.8	1.818	3.247	2.894	3.261	2.894	3.262
10	5	3.33	1.66	1.2	1.05	3.510	1.690	4.470	1.223	4.470	1.223
10	10	3.33	3.33	1.61	1.22	3.506	3.414	4.511	3.218	4.510	3.219

Table 4.14. Different between the ideal concentration and calculated concentration in 1st experiment.

Ideal Concentration		Proposed		Simultaneous equation		Absorb ratio	
Red	**Green**	**Red**	**Green**	**Red**	**Green**	**Red**	**Green**
0.00	1.66	0.043	0.112	0.179	0.635	0.179	0.635
1.66	0.00	0.057	0.071	0.038	0.047	0.038	0.047
1.66	1.66	0.079	0.050	0.208	1.055	0.208	1.055
1.66	3.33	0.199	0.097	0.262	1.259	0.262	1.259
3.33	1.66	0.052	0.057	0.343	1.417	0.343	1.417
3.33	3.33	0.014	0.016	0.424	1.687	0.424	1.687
Average different		0.074	0.067	0.242	1.017	0.242	1.017

Table 4.15. Different between the ideal concentration and calculated concentration in 2nd experiment.

Ideal Concentration		Proposed		Simultaneous equation		Absorb ratio	
Red	**Blue**	**Red**	**Blue**	**Red**	**Blue**	**Red**	**Blue**
0.00	1.66	0.072	0.085	0.039	0.431	0.039	0.432
1.66	0.00	0.197	0.121	1.074	0.376	1.074	0.376
1.66	1.66	0.170	0.233	1.174	0.014	1.174	0.014
1.66	3.33	0.158	0.083	1.234	0.069	1.234	0.068
3.33	1.66	0.180	0.030	1.140	0.437	1.140	0.437
3.33	3.33	0.176	0.084	1.181	0.112	1.180	0.111
Average different		0.159	0.106	0.974	0.240	0.974	0.240

Table 4.16. Reduction of error of 1st experiment by the proposed method from the previous method.

Ideal Concentration		Simultaneous equation		Absorb ratio	
Red	**Green**	**Red**	**Green**	**Red**	**Green**
0.00	1.66	0.136	0.522	0.136	0.522
1.66	0.00	-0.019	-0.024	-0.019	-0.024
1.66	1.66	0.129	1.005	0.129	1.005
1.66	3.33	0.063	1.162	0.063	1.162
3.33	1.66	0.291	1.360	0.291	1.360
3.33	3.33	0.409	1.671	0.409	1.671
Average reduction		0.168	0.949	0.168	0.949

Table 4.17. Reduction of error of 2nd experiment by the proposed method from the previous method.

Ideal Concentration		Simultaneous equation		Absorb ratio	
Red	**Blue**	**Red**	**Blue**	**Red**	**Blue**
0.00	1.66	-0.032	0.346	-0.033	0.347
1.66	0.00	0.877	0.255	0.877	0.255
1.66	1.66	1.004	-0.219	1.004	-0.219
1.66	3.33	1.076	-0.014	1.076	-0.015
3.33	1.66	0.960	0.407	0.960	0.407
3.33	3.33	1.005	0.029	1.004	0.027
Average reduction		0.815	0.134	0.815	0.134

Table 4.18. Percentage average error reduction of 1st experiment.

	Average error of previous	**Average error of proposed**	**Average error reduction**	**Percentage average error reduction**
Red	0.242	0.074	0.168	69.437%
Green	1.017	0.067	0.949	93.386%

Table 4.19. Percentage average error reduction of 2nd experiment.

	Average error of previous	**Average error of proposed**	**Average error reduction**	**Percentage average error reduction**
Red	0.974	0.159	0.815	83.7%
Blue	0.240	0.106	0.134	55.85%

5. Discussion and conclusion

5.1. Discussion

The proposed spectrophotometric method was designed to calculate the concentration of the component in the pure solution case and the multi-component solution case. The proposed method interpolates the concentration by the value at the boundary points to the ideal concentration approximately. It can be used with the limited wavelength of the spectrophotometer or installing the proposed spectrophotometric method into the hand-made device. The proposed method can calculate the concentration of the ideal case as same as the concentration calculated by the previous methods. Furthermore, in the error case, the concentration result of the proposed method is more approximate to the ideal result than the concentration result of the previous method.

Nevertheless, the proposed method is not the perfect method. It cannot analyze the concentration in every case. Furthermore, the proposed method must require many known concentration data as boundary points to calculate the concentration of solution. The accuracy of the proposed method depends on the position and the number of the boundary points. In the case of the known concentration data that the light absorbance of the detector does not alter while the concentration alters, it cannot calculate because there are many outputs by one input. Therefore, the determination can have an error in this case.

For an example, in case of very low molar absorptivity, the increase of the light absorbance is hard to observe. The resolution of this problem is the alteration of the wavelength which the molar absorptivity is changed also. Furthermore, a spectrophotometer can measure the light absorbance only from 0 until 2. The proposed method provides the boundary points to calculate the concentration of solution. In the case that the input is not in the range of the boundary points, the boundary-point calculating will be attached to cover range of input. The boundary points are calculated by the average of the known concentration data. However, the accuracy of the determination in the case that input is out of range of the known concentration data is lesser than the accuracy of the determination in the case that input is in range of the known concentration data.

In the ideal case of the 2 components, the simulation results of the proposed method and the other methods are the same. The determination of the proposed method can change the light absorbance input and the direction of the component. The calculated concentration result is the same even if the light absorbance input and the direction of the component are changed. However, in the real experiment, there are many errors in the deviation of Beer-Lambert's law case. In the case of the proposed method that changes the input light absorbance or the direction of the component, the concentration outputs are difficult to equal in all cases. Furthermore, the determination providing many variables or many times of the calculation has many errors.

The proposed method cannot only calculate the concentration of the components but also it can calculate the light absorbance from the other detectors [73] or other values which relate with the concentration of solution. Although the analysis of the more than 3-component solution was not performed in this thesis, the spectrophotometric method using fuzzy theory can analysis more than 3 components same as the simultaneous equation method.

5.2. Conclusion

The spectrophotometric method is the concentration calculation method by light absorbance. In the ideal case, the calculation results of every method are perfect. However, in the real experiment, there is the deviation of Beer-Lambert’s law in the light absorbance. Therefore, the calculated concentration is affected by the

deviation of Beer-Lambert's law. Therefore, to reduce errors of results in the case of the deviation of the Beer-Lambert's law, we have suggested the novel spectrophotometric method using fuzzy theory.

In section 2, we analyzed the spectrophotometric method. There was the relationship between the light absorbance and the concentration of solution and the causes of the deviation of Beer-Lambert's law. From the analysis, it confirmed that the linear regression analysis is an important part in calculating the concentration of the component by light absorbance. In the multiple component case, the linear regression analysis was used to calculate the molar absorptivity between the concentration and the wavelength or calculate the linear equation between the concentration and the output of each method in the last process. The multi-component spectrophotometric method had 2 main methods. The first method provided all molar absorptivity between the pure solutions and the wavelengths of light source. The second method was the elimination of the value of the disinterest component. Some methods utilized the specific point to make the determination easier. The method that can use in every spectrophotometer without using the specific point was the simultaneous equation method and the absorb ratio method only. From the deviation of Beer-lambert's law, it confirmed that the light absorbance is not proportional to the concentration of solution. Therefore, the spectrophotometric method by the linear regression analysis has an error depending on the deviation of Beer-lambert's law.

In section 3, we suggested the novel spectrophotometric method using fuzzy theory. To calculate the linear regression equation, the light absorbance of known concentration solution must be required. The previous method provides the linear regression analysis to calculate the concentration. Therefore, in the deviation of Beer-Lambert's law case, there are many errors. Thus, the nonlinear approximation method was provided to calculate concentration of the deviation of Beer-Lambert's law case. The proposed method utilized the linear interpolation based on the fuzzy theory to calculate the concentration of solution by the light absorbance. The linear interpolation required the variables of the known concentration solutions as the boundary points. The calculation interpolates the data between the boundary points by the piecewise linear. Therefore, the calculated concentration by the proposed method is approximate to the concentration of the deviation of Beer-Lambert's law. Furthermore, the proposed method can be used with the pure solution and the multi-component solution. In the case of the multicomponent solution, the number of the light absorbance are equal to the number of the component in the mixture solution same as the simultaneous equation method. Furthermore, the calculation of the concentration in the case of the 2-component solution is the bilinear interpolation. It is like the simultaneous equation method that can be used in every spectrophotometer without the specific condition and derivative function. Therefore, the proposed method can be utilized with every spectrophotometer without the specific condition and derivative function.

In section 4, we compared the results of the proposed method with the results of the previous method in the ideal case and the deviation of Beer-Lambert's law case. The calculated concentration in the ideal case is the same in every method. Therefore, the comparison is the deviation of Beer-Lambert's law case in the case of the pure solution and the 2-component solution by simulation.

In the case of the pure solution, the calculated concentration by the proposed method was compared with the calculated concentration by the linear regression analysis. The calculation by the linear regression analysis made the same result with the calculation by the proposed method in the case of the 2 known concentration solutions. The function by the linear regression analysis was the linear equation. When the number of the known concentration solutions increased, all concentrations were reduced. When some concentrations were approximate to the ideal concentration, some concentrations were fairly different from the ideal

concentration. However, the calculated concentration by the proposed method was more approximate to the ideal concentration than the calculated concentration by the linear regression analysis. Furthermore, when the number of the boundary points were increased without the movement of boundary points (2, 3, and 5 known concentration solutions), the calculated concentration was more approximate to the ideal concentration and more without receding from the ideal concentration everywhere.

In the case of the 2 components, the previous methods were the simultaneous equation method and the absorb ratio method that can be used in every spectrophotometer without a specific condition. The standard solution of the absorbance ratio method utilized the molar absorptivity. Therefore, the calculated concentration of both methods was similar. In the case of the 2 known concentration solutions, the calculated concentration between the previous method and the proposed method were similar. When the number of the known concentration solution increased, the average calculated concentration of both components by the previous method did not change too much. The average error of concentration showed that the calculated concentration was slightly approximate to the ideal concentration of solution. In the other hand, the average calculated concentration of both components by the proposed method is significantly approximate with the ideal concentration without receding from the ideal concentration everywhere, when the number of the boundary points increased without dislocation of the boundary points (2, 3, and 5 known concentration solutions).

The average error comparison showed that when the number of the known concentration solution were more than 3, the errors of the average calculated concentrations by the proposed method were reduced higher than the errors of the average calculated concentrations by the previous methods significantly. In the other hand, the errors of the average calculated concentrations by the previous methods were reduced gently. It shows that the proposed spectrophotometric method can calculate the concentration of component to be more approximate to the ideal concentration than the previous spectrophotometric methods. It meant that the proposed method could reduce the error from the previous method. However, the error reduction depended on the deviation of Beer-Lambert's law and the position of the boundary points. Therefore, the reduction of the error cannot be shown clearly. In the experiment, the calculated concentration by proposed method is more approximate to the ideal concentration than the calculated concentration by previous method same as the simulation. It can be observed at the average error of concentration.

Throughout this research, the spectrophotometric method has been analyzed in the pure solution and the multi-component solution. The result of the section 4 can help chemists make a better selection of a spectrophotometric method. Furthermore, in education, it can help the faculties which have limited funds. The faculties are not essential to purchase the expensive high-efficient spectrophotometer for every student. To calculate the concentration by the proposed method, the low-cost spectrophotometer or the hand-made spectrophotometer is sufficient.

5.3. Future study

In this thesis, the proposed method is the external calculation of the concentration of the components for spectrophotometers. Therefore, we need to install the spectrophotometric method in to the microcontroller. The installed device can calculate the concentration of solution directly without the manual spectrophotometric method. Furthermore, the output of the installed device is more approximate to the ideal concentration than the previous manual spectrophotometric method.

In some error cases, it makes the position of the additional boundary points are not in order. Therefore, the determination is this point has error. To reduce this error point, we need to design the fuzzy set and the defuzzification which affects to the other calculated concentration result.

Reference

[1] T. Owen, *Fundamentals of UV-visible spectroscopy*, Hewlett-Packard Company, 1996

[2] A. Hofmann, *Principles and techniques of biochemistry and molecular biology*, Cambridge University Press, pp.477-521, 2010

[3] K. Lawson-Wood, I. Robertson, PerkinElmer and S. Green, *Pharmaceutical assay and multicomponent analysis using the LAMBDA 365 UV/Vis spectrophotometer*, PerkinElmer, 2016

[4] S. Bano, T. Altaf, and S. Akbar, Microcontrolled based spectrophotometer using compact disc as diffraction grid, *Proc. Of SPIE – The international Society for Optical Engineering*, pp.332-336, 2010

[5] Md. Ashfaque-E-Alam, Md. Rakibul Islam and I. Jabeen Faria, Development and validation of a low-cost visible light spectrophotometer, *2017 4th International Conference on Advances in Electrical Engineering (ICAEE)*, 2017

[6] L. Tymecki, M. Pokrzywnicka and R. Koncki, Paired emitter detector diode(PEDD)-based photometry-alternative approach , *The Analyst*, vol.133, no.11, pp.1501-1504, 2008

[7] T.-S. Yeh and S.-S. Tseng, A Low Cost LED Based spectrometer, *Journal of the Chinese Chemical Society*, vol. 53, pp.1067-1072, 2006

[8] S. Kittipanyangam, W. Do, K. Abe, and K. Eguchi, Design of the hand-made light absorbnace measurement device for chemical education, *Internationnal Journal of Innovative Computing, Information & Control*, vol. 12, No.5, pp.1397-1410, 2016

[9] L. Viennot and C. de Hosson, Colour phenomena and partial absorption, *MUSE group (More Understanding with Simple Experiments) in the Physics Education Division (PED) of the European Physical Society (EPS),* 2012

[10] S. Kittipanyangam, P. Rattanachinalai, K. Abe, W. Do, K. Eguchi, Handmade light absorbance measurement device by using cellophane as filter for educational purpose, *Proceedings of the 5th IIAE International Conference on Industrial Application Engineering 2017*, pp.151-158, 2017

[11] S.M. Liu, The development of a portable spectrophotometer for noncontact color measurement, *IEEE Trans. Instrumentation and measurement*, vol.53, no.1, pp.155-162, 2004

[12] D. R. Albert, M. A. Todt, and H. Floyd Davis, A low-cost quantitative absorption spectrophotometer, *Journal of chemical education*, vol. 89, no. 11, pp. 1432-1435, 2012

[13] M.F. DeCamp and A. Tokmakof, Single-shot two-dimensional spectroscopy, *2006 Conference on Lasers and Electro-Optics and 2006 Quantum Electronics and Laser Science Conference*, 2006

[14] S.Kittipanyangam, W.Do, K.Eguchi, Color light sensor device for light absorbance measurement device, *Proceedings of the 14th International Conference on Electrical Engineering/Electronics, Computer, Telecommunications and Information Technology 2017 (ECTI-CON2017)*, pp.1-4, 2017

[15] M. Rohitas, A. Agrawa, A. K Jain, N. K Lariya, A. K Kharya and G. P. Agrawal, Development of the simultaneous spectrophotometric method of mesalazine and prednisolone in same dosage from, *International journal of applied pharmaceutics*, vol.2, no.4, 2010

[16] A. H. Kamal, S. F. El-Malla and S. F. Hammad, A review on UV spectrophotometric method for simultaneous multicomponent analysis, *European journal of pharmaceutical and medical research*,vol.3, no.2, pp.348-360, 2016

[17] V. Vichare, P. Mujgond, V. Tambe and D. S.N., Simultaneous spectrophotometric determination of Paracetamol and Caffeine in tablet formulation, *International Journal of PharmTech Research*, vol.2, no.4, pp.2512-2516, 2010

[18] R. Gondalia, R. Mashru, P, Savalaliya, Development and validation of spectrophotometric methods for simultaneous estimation of Ibuprofen and Paracetamol in soft gelatin capsule by simultaneous equation method, *International Journal of ChemTech Research,* vol. 2, No, 4 pp.1881-1885, 2010

[19] S. S. Chitlange, S. Ranjana,S. B. Wankhede and, A. A. Kulkarni, Spectrophotometric methods for simultaneous estimation of Nimesulide and Drotaverine, *International Journal of ChemTech Research,* Vol. 1, No. 2 pp.135-138, 2009

[20] J. Chaudhary, A. Jain and, V. saini, Simultaneous estimation of multicomponent formulations by UV-visible spectroscopy : An overview, *International research journal of pharmacy,* vol. 2, no. 12, pp.81-83, 2011

[21] A Chandratrey, R sharma, Simulataneous spectrophotometric extimation and validation of three component table formulation containing paracetamol, nimesulide and tizanidine, *Indian jounal of the chemical thechnology*, vol. 17 pp.229-232, 2010

[22] D. M Atole and, H. H Rajput, Ultraviolet spectroscopy and its pharmaceutical applications, *Asian journal of pharmaceutical and clinical research*, vol. 11, no.2, 2018

[23] S. Kus, Z. Marczenko and N. Obarski, Derivative UV-VIS spectrophotometry in analytical chemistry, *Chemia analityczna*, vol.41, no.899,1996

[24] M. Mutasim Elimam, S. Wagiealla Shantier, E. Ahmed Gadkariem, and M. Awadalla Mohamed, Derivative spectrophotometric methods for the analysis and stability studies of colistin sulphate, *Journal of Chemistry*, vol. 2015, 2015

[25] M. Ansari, M. Kazemipour, M. Baradaran and H. Jalalizadeh, Derivative spectrophotometric method for determination of losartan in pharmaceutical formulations, *Iranian journal of pharmacology & therapeutics*, vol. 3, no. 1, pp.21-25, 2004

[26] J. Uddin, *Macro To Nano Spectroscopy,* InTech, pp.253-266, 2012

[27] P. G. Patel, V.M. Vaghela , S. G. Rathi, N.B. Rajgor, V. H. Bhaskar, Derivative spectrophotometry method for simultaneous estimation of rupatadine and montelukast in their combined dosage form, *Journal of young pharmacists*, vol.1, no. 4, pp.354-358, 2009

[28] C. Solanki, N. Patel, V. Patel, D. Patel and R.Vaishy, Development and validation of first order derivative spectrophotometric method for simultaneous estimation of rosuvastatin calcium and aspirin in capsule dosage form, *Scholars research library*, vol. 4, no.3, pp.947-953, 2012

[29] N. M. Bhatt, V. D. Chavada, M. Sanyal and P. S. Shrivastav, Manipulating ratio spectra for the spectrophotometric analysis of diclofenac sodium and pantoprazole sodium in laboratory mixtures and tablet formulation, *The scientific world journal*, vol.2014, 2014

[30] T. S. Belala, H. G. Daabees, M. M. Abdel-Khalek, M. S. Mahrousb and M. M. Khamis, New simple spectrophotometric method for determination of the binary mixtures (atorvastatin calcium and ezetimibe; candesartan cilexetil and hydrochlorothiazide) in tablets, *Journal of Pharmaceutical Analysis*, vol.3, no.2, pp.118-126, 2013

[31] A. M. Mohsen, H. M. Lotfy, A. M. Badawey, H. Salem, and S. Z. Elkhateeb, Application of three novel spectrophotometric method manipulating ratio spectra for resolving a pharmaceutical mixture of the chlorphenoxamine hydrochloride and caffeine, *International journal of pharmacy and pharmaceutical sciences*, vol.5, no.1, 2013

[32] J. Akhtar, J. Prajapati and G. Osman Elhassan, Absorbance ratio and derivative spectroscopy methods for the simultaneous estimation of lornoxicam and eperisone in their synthetic mixture, *Indian Journal of Chemical Technology*, vol.22, pp.333-337, 2015

[33] R. Hajian, N. Shams and I. Kaedi, Application of ratio derivative spectrophotometry for simultaneous determination of naphazoline and antazoline in eye drops, *E-Journal of Chemistry*, vol.7, no.4, pp.150-1538, 2010

[34] R. Hajian, R. Haghighi and N. Shams, Combination of ratio derivative spectrophotometry with simultaneous standard additions method for determination of sulfamethoxazole and trimethoprim, *Asian journal of chemistry*, vol.22, no.8, pp.6569-6579, 2010

[35] P. Maczka, A. Gumieniczek, J. Galeza, R. Pietras, Zero crossing and ratio spectra derivative spectrophotometry for the dissolution tests of amlodipine and perindopril in their fixed dose formulations, *Current issues in pharmacy and medical sciences,* vol. 27, no.2, pp.113-117, 2014

[36] Z. Lin, J. Lui, G. Chen, A new method of fourier-transform smoothing with ratio spectra derivative spectrophotometry, *Fresenius journal of analytical chemistry*, pp.997-1002, 2001

[37] H. W. Darwish, F. H Metwally, and A. El Bayoumi, Novel ratio subtraction and isoabsorptive point methods for determination of the ambroxol hydrochloride and doxycycline in their combined dosage from : development and validation, *Tropical journal of pharmaceutical research,* vol. 14, no. 1, pp. 133-140, 2015

[38] P. Jamdar and D. Meshram, Isoabsorptive point method for the simultaneous estimation of neostigmine methyl sulphate and glycopyrroolate in injectable dosage form, *FS Journal of Pharmacy Research*, vol.5,no.1,2015

[39] P. O. Vardevanyan, V. L. Élbakyan, M. A. Shahinyan, M. V. Minasyants, M. A. Parsadanyan, and N. S. Sahakyan, Determination of the isosbetic point in the absorption spectra of DNA-ethidium bromide complexes, *Journal of Applied Spectroscopy*, vol. 81, no. 6, pp.1060-1063, 2015

[40] S. T avker, P. Kumar P, H. R. Carlon, and M. E. Milham, Isosbestic point : An application for aerosol spectrometry, *Journal of geophysical research*, vol. 102, no. D25, pp. 30,017-30,022, 1997

[41] J. Akhtar, J. Prajapati, G. Osman Elhassan, M. Mujahid, Development & validation of the absorbance ratio method for simultaneous estimation of lornoxicam and eperisone in their synthetic mixture, *Indo Global Journal of Pharmaceutical Sciences*, vol.5, no. 3, pp.225-232, 2015

[42] J. C. Khamar and S. A. Patel, Q-absorbance ratio spectrophotometric method for the simultaneous estimation of rifampicin and piperine in their combined capsule dosage, *Journal of applied pharmaceutical science,* Vol. 2, no.4, 2012

[43] N. G. Shinde and N. H. Aloorkar, Development and validation of UV spectrophotometric method for simultaneous estimation of propranolol hydrochloride and rosuvastatin calcium in bulk drug and pharmaceutical dosage form, *International journal of advances in pharmaceutics,* vol.4 no.5, 2015

[44] P. Pallavi , W. Sagar, C. Praveen.D., Q Absorbance ration and area under curve method spectrophotometric method for the simultaneous estimation of ketoprofen, methyl paraben and propyl paraben in their formulated gel form, *Scholars Research Library*, vol.5, no.8, 2013

[45] G. Singh, D. Kumar, D. Sharma, M. Singh and S. Kaur, Q-absorbance ratio spectrophotometric method for the simultaneous estimation of prednisolone and 5-amino salicylic acid in tablet dosage form, *Journal of applied pharmaceutical science*, vol. 2, no. 6, pp. 222-226, 2012

[46] G. Pandey and B. Mishra, A new analytical Q-absorbance ratio method development and validation for simultaneous estimation of lamivudine and isoniazid, *Hindawi Publishing Corporation ISRN Spectroscopy,* vol. 2013, pp.1-5, 2013

[47] S. S. Saleh, H. M. Lotfy , N. Y. Hassan, S. M. Elgizawy, A comparative study of validated spectrophotometric and TLC- spectrodensitometric methods for the determination of sodium

cromoglicate and fluorometholone in ophthalmic solution, *Saudi Pharmaceutical Journal*, vol. 21, pp.411-421, 2013

[48] A. Samir, H. Salem, M. Abdelkawy, New developed spectrophotometric method for simultaneous determination of salmeterol xinafoate and fluticasone propionate in bulk powder and Seritide diskus inhalation, *Bulletin of Faculty of Pharmacy, Cairo University*, vol. 50, pp.121-126, 2012

[49] E. Shokry, A. E. El-Gendy, M. A. Kawy , and M. Hegazy, Application of double divisor ratio spectra derivative spectrophotometric [DDRS-DS], chemometric and chromatographic methods for stability indicating determination of Moexipril hydrochloride and Hydrochlorothiazide, *Current Science International,* vol.3, no.4, pp.352-380, 2014

[50] R. Hajian and A, Soltaninezhad, The Spectrophotometric multicomponent analysis of a ternary mixture of Paracetamol, Aspirin, and Caffeine by the double divisor-ratio spectra derivative method, *Hindawi Publishing Corporation Journal of Spectroscopy*, vol. 2013, pp.1-7, 2013

[51] H. Nie and H. Chen, Piecewise Linear interpolation algorithm in the high precision electronic system, *The 2018 5th International Conference on Systems and Informatics (ICSAI 2018)*, pp. 65-69, 2018

[52] M. Shio, M. Yanagisawa and N. Togawa, Linear and bi-linear interpolation circuits using selector logics and their evaluations, *2014 IEEE International Symposium on Circuits and Systems (ISCAS)*, pp. 1436-1439, 2014

[53] H. Kim, S. Park, J. Wang, Y. Kim, and J. Jeong, Advanced bilinear image interpolation based on edge features, *2009 First International Conference on Advances in Multimedia*, pp.33-36, 2009

[54] K.T. Gribbon and D.G. Bailey, A novel approach to real-time bilinear interpolation, *Proceedings. DELTA 2004. Second IEEE International Workshop on Electronic Design*, Test and Applications, 2004

[55] Biochrom, Basic UV/visible spectrophotometry, Spectro educational booklet 07, *Biochrom,* 2019

[56] S. J. Tavener and J. E. Thomas-oates, Build your own spectrophotometer, *Education in Chemistry*, pp.151-154, 2007

[57] J. Dufour, *Coefficients of determination*, McGill university, 2011

[58] M. Ahmed Zaid, *Correlation and Regression Analysis*, Organisation of Islamic cooperation, 2015

[59] H. J. Seltman, *Experimental design and analysis*, http://www.stat.cmu.edu/ ~ hseltman/309/Book/ Book.pdf, 2018

[60] J. Mellqvist and A. Rosen, DOAS for flue monitoring – II. Deviations from the Beer-Lambert law for the U.V./visible absorption spectra of NO, NO_2, SO_2 and NH_3, *Journal of quantitative spectroscopy and radiative transfer*, vol.56, no. 2, pp.209-224,1996

[61] Paul Held, Ph.D.,Laboratory Manager, Applications Department, BioTek Instruments, Inc., Winooski, VT USA, Using Phenol red to assess pH in tissue culture media, *Application Note Live Cell Imaging,* BioTek Instruments, Inc, pp.1-7 ,2018

[62] Anatolii V. Omelcheno and Oleksii V. Fedorov,Polynomial regression coefficients estimation in finite differences space, *2015 25th International Conference Radioelektronika (RADIOELEKTRONIKA),* 2015

[63] M. A. Mostafa, Application of regression analysis for predication of voltage collapse in power systems, *2008 12th International Middle-East Power System Conference*, pp.529-535, 2008

[64] J. C. Bezdek, *Pattern Recognition with Fuzzy Objective Function Algoritms*, Plenum Press, New York, 1981

[65] H.J. Zimmermann, *Fuzzy Set Theory and Its Applications*, Kluwer Academic Publishers, 2001

[66] F. Chevrie, *Fuzzy logic*, Groupe Schneider, 1998

[67] S. Kittpanyangam , R. Rubpongse, W. Lok Do and K. Eguchi, One color light absorbance measurement device by fuzzy theory, *Proceedings of the 6th IIAE international conference on industrial application engineering 2018*, pp.35-42, 2018

[68] Y. Bai and D. Wang, Fundamentals of fuzzy logic control – Fuzzy Sets, Fuzzy rules and Defuzzifications, *Advanced Fuzzy Logic Technologies in Industrial Applications,* 2006

[69] K. Eguchi, S. Kurebayashi, H. Zhu, Y. Itoh, A fuzzy-based educational system to assist self-learning for pupils, *International Journal of Innovative Computing, Information and Control*, vol. 4, No. 10, pp. 2441-2450, 2008

[70] K. C. Hung, M. Yin, K. P. Lin, Enhancement of fuzzy weighted average and application to military UAV selected under group decision making, *2009 Sixth International Conference on Fuzzy Systems and Knowledge Discovery*, pp.191-195, 2009

[71] D. Wu, Member, and J. M. Mendel, Ordered fuzzy weighted averages and ordered linguistic weighted averages, *IEEE,* 2010

[72] M. Jiang, X. Yuan, H. Li, and J. Wang, A new fuzzy system based on rectangular pyramid, *The Scientific World Journal*, vol. 2015, pp. 1-11, 2014

[73] S. Kittipanyangam, W. Do, R. Rubpongse, K. Eguchi, Design of a fuzzy-based light absorbance measurement device for chemical education, *Proceedings of the 4th International Conference on Engineering, Applied Sciences and Technology: Exploring Innovative Solutions for Smart Society (ICEAST'2018)*, pp.1-4, 2018

Appendix

WPA CO 7500 Colorimeter

Figure appendix. 1. Spectrophotometer WPA CO7500 colorimeter
(Picture from the WPA CO 7500 Colorimeter manual)

Table appendix.1. Specification of Spectrophotometer WPA CO7500 colorimeter

Wavelength range	440 – 680nm
Standard gelatin filters	440, 470, 490, 520, 550, 580, 590 and 680nm
Bandwidth	40nm
Range	Absorbance –0.3A to 1.99A % Transmission – 0 – 199% T
Accuracy	<±0.05A at 1A using Neutral Density Filters
Repeatability	±0.02A at 1A using cuvettes
Operational modes	Absorbance, Transmission, Kinetics
Cuvette holder	Fixed with drain hole. Accepts 10mm path length semi micro and macro cuvettes or 16mm round tubes. Can accept 10-12mm tubes with optional adapters
Output	0 – 2V for 0 – 2Abs or 0 – 1.99V for 0 –199%T (via 2 x 4mm sockets, ~ 100mV offset in the output voltage) RS232
Power requirements	External power adaptor (110 to 220V, 50/60Hz, 20VA) or internal rechargeable NiMH battery (mains/battery version only)
Approximate dimensions	180 x 150 x 60mm
Weight	0.6kg

Fuzzy function of Visual Basic of the Microsoft excel

This program is the 1 dimension of the fuzzy theory which the membership function is triangle membership function. The calculation of the fuzzy preparation process and the fuzzy analysis process are the same.

```
‘Function name and the variable x=input, y=boundary point of the input, z=boundary point of the output
Function fuzzy7(x, y, z) As Double
‘Variable setting
Dim b As Integer
Dim a As Integer
Dim sum As Double
Dim sum2 As Double
Dim Avg As Double
Dim Avg2 As Double
Dim count As Integer
Dim count2 As Integer

a = 0
count = 0
count2 = 0

‘the count of the number of the boundary point of the output
Do Until Cells(y.Row, y.Column + a) = ""
a = a + 1
Loop

‘The calculation of the boundary point in the case that the input is not in the range of the boundary point
Avg = (Cells(y.Row, y.Column + a - 1).Value - Cells(y.Row, y.Column)) / (a - 1)
Avg2 = (Cells(z.Row, z.Column + a - 1).Value - Cells(z.Row, z.Column)) / (a - 1)

‘Check the boundary point which the last boundary point of input is higher than the first boundary point of input (A(0,0,1st) > A(0,5,1st))
If Cells(y.Row, y.Column + a - 1).Value - Cells(y.Row, y.Column).Value < 0.0001 Then

‘When the input is lesser than the first boundary point of (Example.A1st < A(0,0,1st)), the output data is the first boundary point of the output
    If x.Value < Cells(y.Row, y.Column).Value Then
    fuzzy7 = Cells(z.Row, z.Column).Value
    End If

‘When the input detector is higher than the last boundary point of the input (Example.A1st > A(0,5,1st)), the output is the last boundary point of the output
    If x.Value > Cells(y.Row, y.Column + a - 1).Value Then
```

```
        fuzzy7 = Cells(z.Row, z.Column + a - 1).Value
        End If

'The case excepting the last boundary point is higher than the first boundary point (Example.A(0,0,1st) < A(0,5,1st))
Else

'In the case that the input is lesser than the first boundary point of input
(Example. A1st < A(0,0,1st))
If x.Value < Cells(y.Row, y.Column).Value Then

'Check the difference between the input and the first boundary point of the input
        Do Until x.Value > Cells(y.Row, y.Column).Value - (Avg * count2)
        count2 = count2 + 1
        Loop

'The calculation of the degree of each function (Wi)
        i = (Cells(y.Row, y.Column).Value - ((count2 - 1) * Avg) - x.Value) / Avg
        j = (x.Value - (Cells(y.Row, y.Column).Value - ((count2) * Avg))) / Avg

'The calculation of the defuzzification (S = ∑i=0k SiWi / ∑i=0k Wi) (interpolation)
        sum = i * (Cells(z.Row, z.Column).Value - ((count2) * Avg2)) + j * (Cells(z.Row, z.Column).Value - ((count2 - 1) * Avg2))
        sum2 = i + j
        fuzzy7 = sum / sum2

'End of the case that the input is less than the first boundary point of the input
End If

'In the case that the input is in the range of the boundary points
(Example. A(0,0,1st) < A1st < A(0,5,1st))
If x.Value >= Cells(y.Row, y.Column).Value And x.Value < Cells(y.Row, y.Column + a - 1).Value Then

'Check the position of the input in the range of the boundary point
        For b = 0 To a – 2
        If x.Value >= Cells(y.Row, y.Column + b).Value And x.Value <= Cells(y.Row, y.Column + b + 1).Value Then

'The calculation of the degree of each function (Wi)
        i = (Cells(y.Row, y.Column + b + 1).Value - x.Value) / (Cells(y.Row, y.Column + b + 1).Value - Cells(y.Row, y.Column + b).Value)
```

```
        j = (x.Value - Cells(y.Row, y.Column + b).Value) / (Cells(y.Row, y.Column + b + 1).Value -
Cells(y.Row, y.Column + b).Value)

'The calculation of the defuzzification (S = Σ_{i=0}^{k} S_i W_i / Σ_{i=0}^{k} W_i) (interpolation)
        sum = i * Cells(z.Row, z.Column + b) + j * Cells(z.Row, z.Column + b + 1)
        sum2 = i + j
        fuzzy7 = sum / sum2

'End of the Check the position of the input in the range of the boundary point
        End If
Next b

'End of the case that the input is in the range of the boundary points
End If

'In the case that the input is higher than the last boundary point of the input
(Example.A_1st > A_(0,5,1st))
If x.Value >= Cells(y.Row, y.Column + a - 1).Value Then

'Check the difference between the input and the last boundary point of the input
        Do Until x.Value <= Cells(y.Row, y.Column + a - 1).Value + (Avg * count2) + 0.0001
        count2 = count2 + 1
        Loop

'The calculation of the degree of each function (W_i)
        i = (Cells(y.Row, y.Column + a - 1).Value + ((count2) * Avg) - x.Value) / Avg
        j = (x.Value - (Cells(y.Row, y.Column + a - 1).Value + ((count2 - 1) * Avg))) / Avg

'The calculation of the defuzzification (S = Σ_{i=0}^{k} S_i W_i / Σ_{i=0}^{k} W_i) (interpolation)
        sum = i * (Cells(z.Row, z.Column + a - 1).Value + ((count2 - 1) * Avg2)) + j * (Cells(z.Row,
        z.Column + a - 1).Value + ((count2) * Avg2))
        sum2 = i + j
        fuzzy7 = sum / sum2

'End of the case that the input is higher than the last boundary point of the input
End If

'End of the check of the boundary point
End If
'End of function
End Functionw
```