

Tristimulus Colorimetry Using a Digital Still Camera and Its Application to Determination of Iron and Residual Chlorine in Water Samples

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Tristimulus colorimetry using a digital still camera (DSC) as a colorimeter has been developed. A photograph of a sample and standard solutions was taken simultaneously with the DSC, and it was transferred to a PC. On the PC, the colors of the sample and of the standard solutions were analyzed and L^* (brightness), a^* (red-green component), and b^* (yellow-blue component) values were determined with laboratory-made software. A dedicated light-box containing white-color LEDs as light source was made of white acrylic to make constant exposure at each photograph. Various settings of the DSC, such as exposure mode, white balance, and so on, that affect analytical figures, were studied with determination of iron with 1,10-phenanthroline. This method was successfully applied to the determinations of iron in a river water sample and of residual chlorine in tap water samples with *N,N*-diethylphenylenediamine (DPD).

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Introduction

Simple and not so expensive analytical methods and/or instruments are required in many fields. They can be applicable to various purposes, such as screening, field analysis, and education. One widely used technique is a visual method including test papers,¹ pack tests,² and so on. It has advantages that no special equipment is required and its operation is usually simple. But it is susceptible to the defect that the difference of sensibilities among operators causes errors if particular devices are not included in the methods. Hence simple analytical instruments for the specific purposes have also been developed extensively and are commercially available. We also developed a portable potentiostat³ and a colorimeter⁴ for field analysis. Another way to develop such analytical methods is to use mass products as analytical instruments. Because of their large yields, their costs are far lower than those of analytical instruments. Using an image scanner as a densitometer for the thin-layer chromatographic determination is a typical example.⁵

From this point of view, we have developed tristimulus colorimetry using a digital still camera (DSC) as a colorimeter. Tristimulus colorimetry is widely used to control the quality of foodstuffs and the colors of printing results. Its first application to chemistry was the characterization of color change in titrimetry⁶ and it was then applied to the determination of various analytes with solid phase colorimetry.^{7,8} It is superior to ordinal colorimetry thanks to its wider dynamic range and

simultaneous multi-component determination ability.⁹ To map the color of objects in color space, either a photoelectric tristimulus colorimeter or a spectrophotometer has been used. The former has three wide-band filters corresponding to the spectral sensitivity curves of human eyes. A charge-coupled device (CCD) of a DSC has also three filters. Although the values obtained by a DSC may be different from those obtained by such a colorimeter because of the difference of their filters, the DSC can be used as a simple tristimulus colorimeter. Actually, the characterization of a DSC as a tristimulus colorimeter was evaluated in detail,¹⁰ and its application to the determination of shape and color of fruits was developed.¹¹ But as far as we know, its application to the chemical analysis has not been studied yet.

In this work, we have developed the software to get color difference of samples from photographs and the light box containing white-color light-emitting diodes (LEDs) for constant exposure. As an example, determination of iron with 1,10-phenanthroline was studied and the effects of several exposing parameters to analytical figures were investigated. Then, as real applications, iron in a river water sample and residual chlorine in tap water samples¹² were determined.

Experimental

Cameras and light box

The DSCs used were Canon PowerShot S45 and Canon IXY Digital L which have 4 million pixels, respectively. Because the former has manual exposure mode, it was used to evaluate this method. A schematic diagram of the light box is shown in

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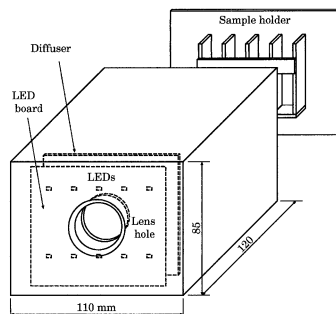


Fig. 1 Schematic diagram of the light box. The LED board and the light diffuser were built in the box.

Fig. 1. It was made of white acrylic whose thickness was 2 mm. At one end, a hole for the lens was opened and 8 or 10 white color LEDs (Nichia NSCW100, surface-mount type, 2×3 mm) were placed around it. The LED driver was made with a dedicated IC (Monolithic Power Systems MP1521) and powered by a 6 V DC wall adapter or 4 AA dry cell batteries. At the other end, a sample holder was set which could accommodate 7 test tubes (Fisher Scientific 14-961-25, 75 mm length and 10 mm o.d.) or 4 standard glass cuvettes (Fujiwara Scientific MG-10, optical path length 10 mm). The dimensions of the box were $110 \times 85 \times 120$ mm (width \times height \times depth) and they were determined from the angle of view of the cameras and the camera-to-sample distance.

Software

Color-analysis software was built with Visual Basic ver. 6.0. Freely available library, "imgctl.dll" which can be downloaded from <http://www.ruche-home.net/>, was linked to the software to display the photograph and to get the standard red, green and blue (sRGB) values of a particular point on it. The user could specify by mouse the regions whose color values were to be determined. Their areas could be changed according to the size of the sample. The sRGB values of all the pixels in the selected region were obtained and averaged; then they were converted to CIE $L^*a^*b^*$ values. The L^* means brightness and larger value is brighter. The a^* means red-green components and a positive value means reddish and a negative one greenish. Similarly the b^* means yellow-blue components. The following equations were used to convert sRGB to $L^*a^*b^*$.

At first, sRGB (R, G and B) values are converted to linear RGB (R, G and B):

$$\begin{aligned} R &= (R/255)^{2.2} \\ G &= (G/255)^{2.2} \\ B &= (B/255)^{2.2} \end{aligned} \quad (1)$$

Then linear RGB values were converted to XYZ values:

$$\begin{aligned} X &= 0.3933R + 0.3651G + 0.1903B \\ Y &= 0.2123R + 0.7010G + 0.0858B \\ Z &= 0.0182R + 0.1117G + 0.9570B \end{aligned} \quad (2)$$

Finally, XYZ values are converted to $L^*a^*b^*$ values:

$$\begin{aligned} L^* &= 116(Y/Y_n)^{1/3} - 16 \\ a^* &= 500[(X/X_n)^{1/3} - (Y/Y_n)^{1/3}] \\ b^* &= 200[(Z/Z_n)^{1/3} - (Y/Y_n)^{1/3}] \end{aligned} \quad (3)$$

where $X_n = 0.98072$, $Y_n = 1.0000$, and $Z_n = 1.1823$. In actual

measurements, a reference region was selected by mouse clicking and L^* , a^* , and b^* values were determined. After that, the differences of values from the reference region, ΔL^* , Δa^* , and Δb^* were calculated by clicking on the desired regions. This software was run on a PC (Sony VAIO U101, Celeron 600 MHz, RAM 512 MB, HDD 30 GB, Windows XP Home edition).

Reagents and samples

All of the reagents used were of analytical grade and deionized and distilled water was used throughout all experiments. For iron determination, an iron(III) standard solution (1000 mg dm^{-3}) was prepared by dissolving 0.723 g of iron(III) nitrate nonahydrate in 10 cm^3 of 1 mol dm^{-3} hydrochloric acid and by diluting to 100 cm^3 with water. Other solutions, such as 1,10-phenanthroline (0.4%), hydroxylammonium chloride (1%), and sodium acetate (3 mol dm^{-3}) were prepared by dissolving the proper amount of each reagent in water. The river water sample was collected from Nigori river which flows through Kofu City, Yamanashi Prefecture, Japan. After collection, the sample was acidified with 0.1 mol dm^{-3} hydrochloric acid and then filtrated by a 25 mm o.d., $0.45 \mu\text{m}$ pore size, hydrophilic PTFE membrane filter (Millipore, Ominipore, Type JH), or acidified after filtration. For residual chlorine determination, DPD reagents, which contained 4% of DPD and 96% of anhydrous sodium sulfate and potassium iodide were purchased from Wako Pure Chemical and were used as received. A phosphate buffer solution was prepared by adjusting the pH of 100 cm^3 of 0.2 mol dm^{-3} potassium dihydrogen phosphate to 6.5 with 0.2 mol dm^{-3} sodium hydroxide and by adding 0.13 g of 1,2-cyclohexanediaminetetraacetic acid monohydrate. As a colorimetric standard, a C.I. Acid Red 265 (disodium 1-(4-methylbenzenesulfonamide)-7-(2-methylphenylazo)-8-hydroxy-3,6-naphthalenedisulphonate) solution was used. This solution corresponding to 50 mg dm^{-3} chlorine was prepared by diluting 32.9 mg of C.I. Acid Red 265 in 100 cm^3 of water. It was stored in a refrigerator and was used within 6 months. The tap water samples were collected from our laboratory in University of Yamanashi and the apartment in Kofu City, Yamanashi Prefecture, Japan. They were kept flowing for 15 min before sampling.

Other equipments

For comparative studies, a spectrophotometer (Shimadzu UV160) and an LED-base, chlorine colorimeter (Hanna HI-93711) were used; the former was for iron determination and the latter was for chlorine determination.

Procedures

For iron determination, 5 cm^3 each of a river water sample or $0 - 2.0 \text{ mg dm}^{-3}$ iron standard solution was taken in a 10 cm^3 measuring flask. Then 1 cm^3 each of 1% hydroxylammonium chloride and 0.2% 1,10-phenanthroline and 1.5 cm^3 of 4 mol dm^{-3} sodium acetate solutions were added successively. After diluting to the mark with water, the resultant solutions were transferred to the test tubes or sample cuvettes and they were set into the light box. The photograph for the standard solutions and sample solution was taken simultaneously and the calibration curve was made for each measurement using Δb^* values.

For residual chlorine determination, 0.5 cm^3 of the phosphate buffer solution and 0.1 g of DPD reagent was taken into a 10 cm^3 measuring flask. Then 5 cm^3 of a sample was added and water was added to the mark. After being mixed vigorously, the solution was transferred to the cuvette. Its photograph was taken together with colorimetric standard solutions corresponding to 0 to 1.0 mg dm^{-3} of chlorine within 1 min. The concentration of free chlorine in the sample was determined

Table 1 Calibration curves under various exposure conditions

Parameter	Equation ^a	<i>r</i> ^b
Normal	$\Delta b^* = 10.1C_{Fe} + 5.2$	0.997
EV ^c + 1	$\Delta b^* = 10.6C_{Fe} + 4.2$	0.997
EV - 1	$\Delta b^* = 7.9C_{Fe} + 4.1$	0.991
WB ^d daylight	$\Delta b^* = 10.0C_{Fe} + 6.3$	0.994
WB fluorescent light	$\Delta b^* = 9.8C_{Fe} + 6.0$	0.995
Color effect vivid	$\Delta b^* = 12.5C_{Fe} + 6.2$	0.998
Color effect neutral	$\Delta b^* = 7.7C_{Fe} + 4.1$	0.994
Program auto	$\Delta b^* = 9.8C_{Fe} + 5.3$	0.996
IXY Digital L, auto	$\Delta b^* = 9.6C_{Fe} - 0.9$	0.998

PowerShot S45 camera was used except for the last.

a. C_{Fe} is concentration of iron in mg dm^{-3} .

b. Correlation coefficient.

c. Exposure value.

d. White balance.

from the Δa^* value of the photograph. Next, 0.1 g of potassium iodide was added into the solution, which was then shaken and kept standing for 2 min. After that, its photograph was taken again and the concentration of total residual chlorine was determined from the Δa^* value.

Results and Discussion

Effect of exposure conditions

Since the DSC had various modes for exposure, their effects to the determination results were investigated with the PowerShot S45 camera which had manual exposure mode. Exposure conditions were fixed as follows, unless otherwise described: shutter speed, 1/45 s; aperture, f2.8; white balance, auto; color effect, normal; and ISO sensitivity, 50. In these experiments, test tubes were used as sample cuvettes. The calibration curves obtained under this and other conditions were linear from 0 to 2.0 mg dm^{-3} of iron and are tabulated in Table 1. At first, exposure value was changed. While there was no effect to calibration curve at exposure compensation +1 (overexposure), less sensitivity and worse linearity were obtained when it was -1 (underexposure). Next, the effect of white balance was examined. The settings of "daylight" and "fluorescent light" did not affect the slopes of calibration curves. Similarly, no effect of exposure controls (manual and program) was found. On the other hand, the color effect modes (vivid, normal, and neutral) made noticeable differences to the slopes. Higher sensitivity was obtained at vivid mode and lower sensitivity at the other modes. Thus most of the parameters did not affect the determination performance. This is because the calibration curve was built with differences from the value of reference region and therefore the effects of various modes would be corrected. Perhaps underexposure would be beyond the latitude of this correction and would cause the worse linearity. The color effects emphasize or deemphasize hue, and they could change the sensitivity without deterioration of the linearity.

Effect of cameras

The effect of cameras on the determination performance was investigated. It was found that the manual exposure mode was not necessary. So a less expensive camera, IXY Digital L, was tested and compared to the PowerShot S45. The results are also tabulated in Table 1. Slightly less sensitivity was obtained. The value of intercept was different from that of S45. This is because the light boxes used were different and the color values

Table 2 Calibration curves under various conditions using IXY Digital L camera

Parameter	Equation ^a	<i>r</i> ^b
Test tubes	$\Delta b^* = 9.6C_{Fe} - 0.9$	0.998
Cuvettes (transparent)	$\Delta b^* = 13.9C_{Fe} + 0.7$	0.998
Cuvettes (frosted)	$\Delta b^* = 12.6C_{Fe} + 4.4$	0.997
8 LEDs	$\Delta a^* = 6.6C_{Fe} - 1.2$	0.999
	$\Delta b^* = 12.6C_{Fe} + 4.4$	0.997
	$\Delta L^* = -6.4C_{Fe} - 9.3$	0.97
10 LEDs	$\Delta a^* = 6.7C_{Fe} - 0.5$	0.999
	$\Delta b^* = 12.9C_{Fe} + 4.1$	0.997
	$\Delta L^* = -7.2C_{Fe} - 8.2$	0.993
Compensation for cell position	$\Delta b^* = 12.1C_{Fe} + 5.3$	0.999
	$\Delta L^* = -6.5C_{Fe} - 7.2$	0.997

a. C_{Fe} is concentration of iron in mg dm^{-3} .

b. Correlation coefficient.

of their reference regions were different. Further experiments were performed with IXY for its simple operation.

Effect of sample cuvettes

At first, less expensive test tubes were used as sample cuvettes. Due to their round surfaces, the reflection of light occurred and the bright region could not be used for determination. So the standard glass cuvettes for colorimetry were tested. To reduce the reflection, not only transparent sides but also frosted sides were evaluated. As expected from their optical path length, sensitivity values were improved when the glass cuvettes were used. Although the transparent side of cuvette gave a better sensitivity than the frosted side, it gave far larger reflection of the light. Those results are tabulated in Table 2. The frosted side of the cuvette was used in further experiments.

Effect of the number of LEDs

Since the homogeneity of the illumination is critical to the determination performance, the number of LEDs, 8 and 10, were tested. These results are also listed in Table 2. Regarding the calibration curve for Δb^* and Δa^* , no distinct difference was observed but the linearity for ΔL^* was improved with 10 LEDs.

Effect of the sample position

It was supposed that the camera lens may cause a drop in brightness at the edges of photograph in addition to inhomogeneity of illumination. So the effect of the sample position was checked by measuring the same standard solutions (0 and 2 mg dm^{-3}) for all the positions simultaneously. The results are shown in Fig. 2. The number 1 is the leftmost side and 4 is opposite. As can be seen from Fig. 2, the values of ΔL^* and Δb^* were severely affected by the position, though those of Δa^* were not. The difference between the mean value of two center positions and that of two borders was used to compensate for the effect of the sample position. The equations for calibration curves of ΔL^* and Δb^* after the compensation are tabulated in Table 2. By the compensation for the position, the linearity was improved.

Application to the determination of iron in river water

This method under the above conditions has been applied to the determination of iron in river water samples. The results by this method before and after the position compensation, and by the spectrophotometer are listed in Table 3. The compensated result agreed well with the latter. The detection limits calculated from 3σ of the blank were 0.2 mg dm^{-3} for this

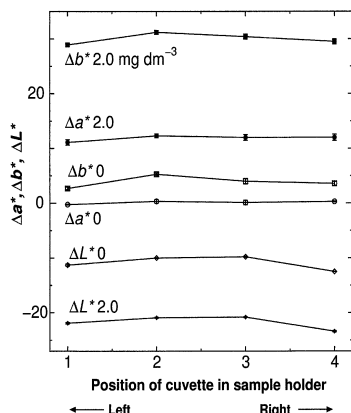


Fig. 2 Effect of the cuvette position to ΔL^* , Δa^* , and Δb^* values. Position 1 means the leftmost and 4 is the rightmost.

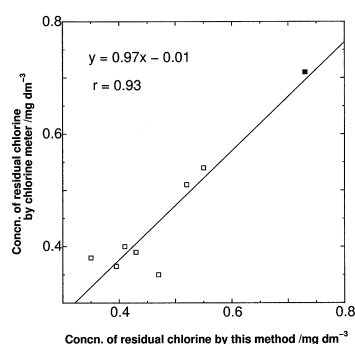


Fig. 3 Correlation between the determination results of free chlorine in tap water obtained by this method and by the chlorine meter. A filled square (■) is a result of on-site analysis.

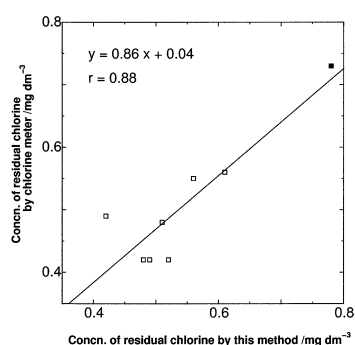


Fig. 4 Correlation between the determination results of total residual chlorine in tap water obtained by this method and by the chlorine meter. A filled square (■) means the same as in Fig. 3.

method and 0.1 mg dm^{-3} for the spectrophotometer. Slightly worse but enough sensitivity was obtained.

Application to the determination of residual chlorine in tap water

This method was also applied to the determination of the free and total residue chlorine in tap water samples. Determination by a commercially available residual chlorine meter which was based on DPD colorimetric method was also performed and the results were compared. The correlation between the results obtained by two methods is shown in Fig. 3 for free chlorine and in Fig. 4 for total residual chlorine. The filled squares are

Table 3 Determination results of iron in a river water sample by this method and by a spectrophotometer ($n = 5$)

Method	Iron found/ mg dm^{-3}	
	Filtrate/acidify	Acidify/filtrate
This method (Δb^* , raw)	0.88 ± 0.03	1.46 ± 0.02
This method (Δb^* , compensated)	0.82 ± 0.03	1.42 ± 0.02
Spectrophotometer	0.81 ± 0.03	1.38 ± 0.02

the results obtained by on-site analysis at the apartment. Compared to chlorine meter, slightly smaller but fairly good results were obtained by this method in higher concentration range, including those of on-site analysis.

Conclusions

The digital still camera was used as a tristimulus colorimeter and it was applied to determine iron in a river water sample and residual chlorine in tap water samples. The determination performance was comparative to that of conventional analytical instruments. To improve accuracy, or to be applied to solid-phase colorimetry, one should redesign the light box to attain more homogeneous illumination. In preliminary study, we used the mobile phone built-in camera (from 1.3 to 2.0 million pixels) to determine iron in river water and obtained satisfactory results. If a program for the mobile phone to analyze color is developed, more convenient determination will be realized.

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