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## Determination of Chromium and Iron Using Digital Image-based Colorimetry

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### Abstract

Recent advance in digital imaging has made a high-resolution photo produced from digital camera more affordable. The use of digital images comprises current opportunity to develop fast and low-cost colorimetric detections for quantitative chemical analysis. The method employs prime color space RGB (red–green–blue) with individual values varying from 0 to 255. RGB data was extracted from a square-homogenous cropped digital images using Matlab software (Mathworks Inc., USA) to construct calibration curve in quantitative determination. We compare two different methods to determine the concentration of chromium (Cr) and iron (Fe). The first method is based on simple linear regression (SLR) of individual color R, G or B. The second method is based on partial least square (PLS) of all three colors R, G and B. Accuracy of the method was validated using UV-visible spectrophotometry as a reference method. We found that accuracy of the method was better than 2.5% with precision less than 1.4% for both chromium and iron.

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## 1. Introduction

Chromium is a transition metal that occurs mainly in Cr (III) and Cr (VI) oxidation states. Although Cr (III) is an essential nutrient, Cr (VI) is well known for its extreme toxicity to the living things. Hexavalent chromium is more soluble in water and its toxicity is 1,000 times higher than Cr (III) [1]. Chromium is a naturally occurring heavy metal found in small quantities associated with other transition metals, particularly iron. Iron is essential to almost all living things, from microorganisms to humans; it is vital to the processes by which cells generate energy. Its concentration in aquatic environment is greatly affected by its speciation. Iron (II) is more soluble than iron (III) in water. Modern oxygenated seawater contains approximately 1-3 ppb of iron. Rivers contain approximately 0.5-1 ppm of iron, and groundwater contains 100 ppm. Iron is the second most abundance of metals on the Earth's crust [2, 3]. Giving its availability and physical strength, Fe is often used in various industry and household manufacturing. Due to their extensive use in manufactured processes, big quantities of chromium and iron compounds are discharged into the environment and they often eventually contaminate the environments. In this context, the determination of chromium and iron in environmental samples, such as river, is important to evaluate the degree of contamination in a given site.

Ideal routine monitoring of chromium and iron concentration in river water must be done using a fast and low-cost, but reliable analytical method. Concurrently with the recent development of digital imaging, digital image-based colorimetry has been used as an alternative for simple determinations in analytical sciences [4, 5]. The methods employ the red–green–blue (RGB) color space and/or its derivatives, such as hue and saturation. The digital-image data are obtained from a scanner [4, 6], a webcam [7], a digital camera [8, 9] and a mobile phone [10] along with charge-coupled devices (CCD) [11, 12] or complementary metal-oxide semiconductor (CMOS) sensors [10, 13]. The color and intensity of digital image are usually 24 bit data (8 bit R + 8 bit G + 8 bit B) forming an additive color space, in which R, G, and B lights are added together in various combinations to reproduce a broad range of colors. By using combination of R, G, and B intensities, many colors can be displayed. The intensity of each color has 256 levels (from 0 to 255). In this color scheme, 16,777,216 colors are obtained ( $256^3$  or  $2^{24}$ ). The value of R = 0, G = 0, B = 0 refers to pure black while R = 255, G = 255, B = 255 is pure white. With this system, unique combinations of R, G and B values are allowed, providing for millions of different hue, saturation, and lightness [14]. These extensive dynamic colors of images provide the database for quantitative analysis. The goal of this study is to propose a simple and robust analytical method employing digital images-based colorimetry for determination of Cr (VI) and Fe (III) in aquatic samples. We compared two different methods of image data processing; (1) based on simple linear regression (SLR) of individual color R, G or B, and (2) based on partial least square (PLS) of all three colors R, G and B.

## 2. Materials and Method

### 2.1. Materials

All reagents used were of analytical grade, all purchased from Merck and used without further purification. Distilled water was obtained from a glass double-distiller and used to prepare all solutions. Stock solution of Cr (III) and Fe (III) were prepared by dissolving appropriate amount of  $K_2Cr_2O_7$  and  $Fe(NO_3)_3 \cdot 9H_2O$ , respectively. Quantitative analysis was conducted using colorimetric reagent of 1,5-diphenylcarbazide (DPC) for Cr (III) and potassium thiocyanate (KSCN) for Fe (III).

### 2.2. Apparatus and Software

Digital Single Lens Reflex (DSLR) camera, with the characteristics of Table 1, was used to record pictures for digital image-based colorimetry. A Thermo Scientific UV–Vis spectrophotometer, model Genesys 20, was used as a reference instrument for the quantitative analysis. Photoshop (Adobe Systems Inc.), Matlab (Mathworks Inc.), Excel (Microsoft Inc.) and Minitab (Minitab Inc.) were used for digital image data processing.

Table 1. Characteristics of the digital camera for Cr and Fe determination

Digital camera	D100 DSLR, Nikon Corporation
Camera maximum resolution	6 megapixels
Camera sensor	CCD, 23.7 x 15.6 mm
Conditions to take pictures	
Exposure mode	Aperture priority, F-stop f/11
Focal length	62 mm
Metering mode	Pattern
White balance	Manually preset
Image format	JPEG, 3008 x 2000 pixels (L)
Color representation	sRGB (standard RGB)
Softwares for image computation	
Transfer image to computer	Photoshop CS4
RGB color measurement	Matlab 7.0
Image data processing	Excel 2007
Image data processing	Minitab 16.0

### 2.3. Photographic Procedure

Standard and sample solutions were placed in spectrophotometric quartz cells of 1 cm optical path in a white background opaque cabinet to maintain the same environmental light and photographic conditions. Detail camera set up is shown in Table 1. Digital images were transferred to a computer using Adobe Photoshop software. Colors were obtained from a representative square region of approximately 1000 pixels located in each spectrophotometric cell (Figure 1a). The average RGB values were measured with Matlab's image processing tool box. Digital image capture and Matlab processing of sample were performed in 4 replicates. Further analysis of RGB data were made by Microsoft Excel and Minitab for simple linear regression (SLR) and partial least square (PLS), respectively.

### 2.4. Reference Method

The reference spectrophotometric procedure was performed using an appropriate complex reagent. Colorimetric determination of chromium is based on reaction of Cr (VI) ion with 1,5-diphenylcarbazine (DPC) producing red-violet colored complex with a maximum absorption of 540 nm. Determination of iron is based on reaction of Fe (III) ion with potassium thiocyanate (KSCN) producing orange-red colored complex with a maximum absorption of 480 nm. The analysis of each sample was performed in triplicate, and the concentrations were calculated from the standard calibration curve.

## 3. Results and Discussion

### 3.1. Quantification of Digital Image

Digital image from the camera was saved in a compact flash card and transferred to the computer using card reader with Adobe Photoshop software. The average R, G and B color values were analyzed using Matlab software. The specific image area was selected as shown in Figure 1a in a black square. We have also tried to use RGB color values from a single pixel that results in poor accuracy and, hence, selection of specific area consisting of multiple pixels is important to obtain an accurate result. Table 2 shows the average R, G and B color values for various concentrations of Cr (VI) and Fe (III), respectively. The plot of RGB values against concentration produces a hyperbolic trend. In order to obtain a linear line for further simple usage, such as standard calibration curve, the initial RGB color values were converted to log ratios following the Lambert-Beer law derivation formula as follow:

$$I_R = \log \frac{R_0}{R_s}$$

$$I_G = \log \frac{G_0}{G_s}$$

$$I_B = \log \frac{B_0}{B_s}$$

$I_R$ ,  $I_G$  and  $I_B$  are the effective intensity for red, green and blue, respectively.  $R_0$ ,  $G_0$ ,  $B_0$ , and  $R_s$ ,  $G_s$ ,  $B_s$  are the red, green and blue color values of blank and sample, respectively. In this respect, digital camera functions as a spectrophotometer analyzing the light passing through the Cr (VI) solution. It should be noted that the term ‘intensity’ here is the number of R, G and B color values of digital image and, thus, differ from that is used in UV-Visible spectrophotometry. The logarithmic conversion of RGB value against various concentrations is shown in Figure 2 and 4 for Cr (VI) and Fe (III), respectively.

Table 2. Color value of Cr (VI) and Fe (III) for different concentration solution

Cr (mg/L)	Blank	0,1	0,2	0,4	0,8
Red	218	199	193	191	182
Green	240	205	181	145	89
Blue	247	241	235	224	196
Fe (mg/L)	Blank	0,2	0,8	1,6	3,2
Red	157	160	159	155	153
Green	175	180	165	149	114
Blue	174	180	160	131	101

Figure 1 shows the typical histogram of the colored Cr (VI) obtained from Adobe Photoshop software. The centered-position of histogram depicts a good exposure of image recording process. Magenta color shown in the histogram is an overlap area between red and blue colors. Green color position is located at the most left side of the histogram, followed by red and blue colors. This result shows that Cr (VI) solution absorbs green ( $\lambda \sim 530$  nm) color more than red ( $\lambda \sim 690$  nm) and blue ( $\lambda \sim 460$  nm) colors. As a consequence, the transmitted red and blue colors, that are slightly absorbed, eventually produced violet ( $\lambda \sim 410$  nm) color of Cr (VI) solution. In other words, the violet color of complex Cr (III) is produced by additive mixing of red and blue colors in RGB color space ( $R=255$ ,  $G=0$ ,  $B=255$ ). Dilute Fe (III) – SCN complex produced orange ( $\lambda \sim 460$  nm) color rather than red color; that usually occurred in a concentrated Fe (III) complex (Figure 3). As shown in the histogram (Figure 3) and Figure 4, blue color is the most absorbed light for Fe (III) complex, in contrast to Cr (VI) complex. No overlap histogram between green and red colors is coincident with slope order trend in the Plot of RGB effective intensity against Cr (VI) concentration (Figure 4). Further exploration of histogram with appropriate different computation for the quantitative analysis assured an interesting result [15].

Figure 2 shows plot of RGB effective intensity against various Cr (VI) concentrations. The best sensitivity (highest slope of the calibration equation) for violet Cr (VI) complex was green color. This result is consistent with histogram showing the green color as the most absorbed light. RGB effective intensity for various Fe (III) concentrations is shown in Figure 4 with blue color that produced better sensitivity than green and red colors did.

### 3.2. Analytical Performance and Application

Precision of the proposed method was evaluated by replicating analysis of image capture and RGB calculation from the same sample. Relative standard deviation (RSD) was calculated to be less than 1.37% and 1.31% for Cr (VI) and Fe (III), respectively. These results show a good repeatability of the proposed method. As described previously, the best sensitivity for Cr (VI) and Fe (III) using simple linear regression determination was achieved by green and blue colors, respectively. Slopes of the calibration curve were 0.548 and 0.082 for Cr (VI) and Fe (III), respectively (Figure 2 and 4). Lower Fe (III) sensitivity than Cr (VI) was probably due to less intensity of the

resulted color of Fe (III) - SCN complex. It could be improved using a different complex reagent for Fe, such as 1, 10-phenantroline.

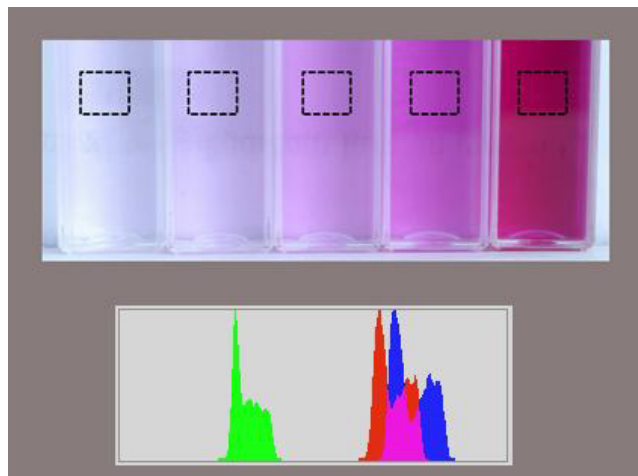


Fig. 1 Digital image of colored Cr (VI) in various concentration from 0 to 0.8 mg/L and its typical histogram

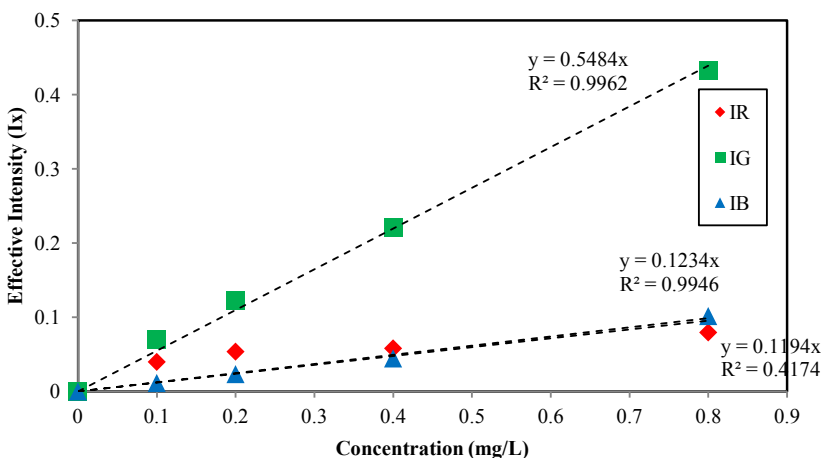


Fig. 2 Plot of RGB effective intensity against Cr (VI) concentration

Simple linear regression was tested using Microsoft Excel to provide the easiest image calculation as it is commonly done using standard calibration curve on UV-visible spectrophotometry. Only green and blue colors were used in image quantification of Cr (VI) and Fe (III), respectively. In contrast, partial least square (PLS regression) calculated using Minitab software is a more sophisticated calculation method that bears some relation to principal components regression [16]. In PLS, all of RGB colors, which were treated as multivariate data, were analyzed to produce a new linear regression to calculate the concentration of Cr (III) and Fe (VI) using all three variables of R, G and B colors. The resulted equations were  $[Cr] = -0.00219R + 0.474678G + 0.52743B$  for Cr (VI), and  $[Fe] = 7.03915R + 7.990521G + 6.140307B$  for Fe (III).

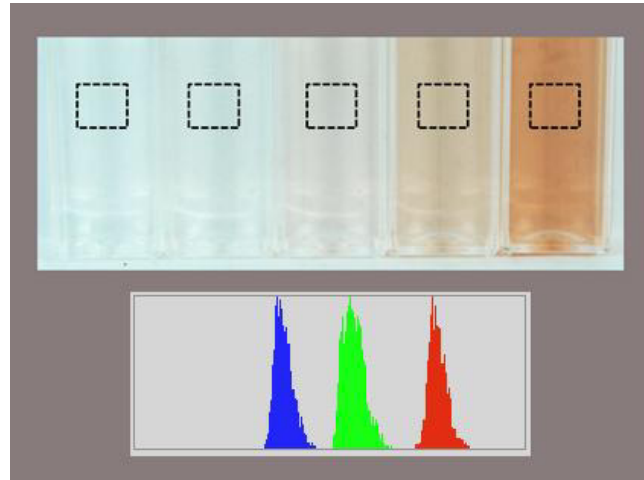


Fig. 3 Digital image of colored Fe (III) in various concentrations from 0 to 3.2 mg/L and its typical histogram

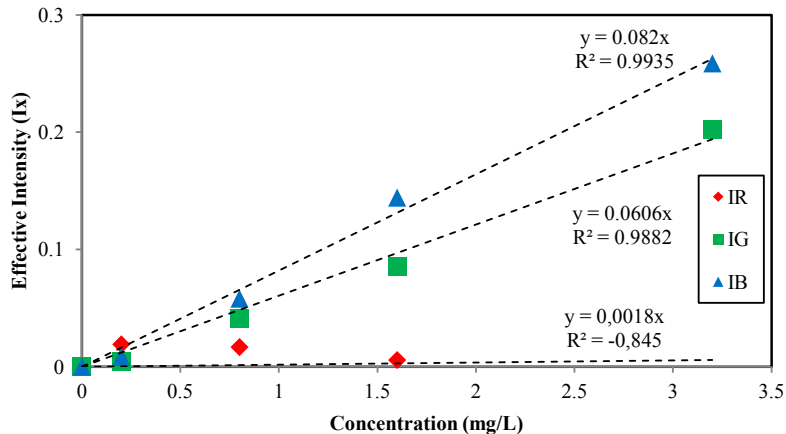


Fig. 4 Plot of RGB effective intensity against Fe (III) concentration

The method was applied to synthetic solution with known Cr (VI) and Fe (III) concentrations. Accuracy of the proposed methods was assessed using UV-visible spectrophotometry as a reference method. The percentage relative error  $\left(\frac{X_e - X_c}{X_c} \times 100\right)$  in the analysis of a known concentration of Cr (VI) and Fe (III) solution ( $X_c = 0.6$  mg/L), ( $X_e$ , result of SLR or PLS) was found to be better than 2.5% for both metals. Although accuracy of PLS is better, SLR image quantification was still acceptable, with no significant statistical differences at a 95% confidence level. One disadvantage of the proposed method is relatively insufficient low limit of detection. Image-based colorimetry detection limit is comparable to that of UV-visible spectrophotometry, which is not good enough to detect low concentration of heavy metals in unpolluted river samples or other environment samples. However, detection limit of the proposed method can be improved by applying additional step before the analysis, such as co-precipitation, ion exchange or chelating resin pre-concentration [17].

#### 4. Conclusion

A fast and low-cost colorimetric method for quantitative analysis of Cr (VI) and Fe (III) in aquatic samples has been proposed. The suggested method also offers good precision, less chemical-reagent usage, simple procedure and unsophisticated instrument. Furthermore, the easiest image quantification using simple linear regression shows sufficient precision and accuracy, comparable with more sophisticated image quantification of partial least square and with more expensive method of UV-visible spectrophotometry.

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