Optimization of digital image-based colorimetric test for the detection of iron in water

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Abstract

In this study, digital image analysis was applied to the colorimetric reaction for iron (II) detection. The reaction condition between iron (II) and 1, 10-phenanthroline was determined to maximize the red-orange complex product. Each color test was photographed by a digital camera with a complementary metal oxide semiconductor (CMOS) image sensor. Adobe Photoshop 11.0 Software was employed to analyze the image color intensity in terms of a red green blue (RGB) value (0-255). The RGB intensity was proportional to the reaction product concentration. The results obtained from the developed method were compared with those from the standard method of UV-vis spectrometry. The optimum condition was found at 5 min reaction time, 0.9 g/L 1, 10-phenanthroline solution, 0.25 M ammonium acetate buffer solution and pH 3.25. With 0.67 – 1.60% difference from the real concentration, this developed method showed the possibility to use as the screening test for the iron (II) in water.

Keywords: Digital image analysis, Iron, RGB color; Colorimetry
1. Introduction

Iron is the fourth most abundant element in the earth’s crust and naturally found in divalent and trivalent states (Rabinovich, 2000). It is an essential element for various kinds of creatures in the animal and plant kingdom, playing a vital role in a variety of biochemical processes and constituting one of the most essential micronutrients. Consumption of iron deficient food samples is the most common cause of anemia occurring during infancy, pregnancy and adolescence, while its intake over and above its certain level, for example, more than 0.3 ppm in drinking water, the toxicity is appeared (Pytlakowska & Feist, 2011).

The accurate and sensitive determination of trace elements are the important part of analytical chemistry studies (Duran, Tuzen, & Soylak, 2010). The detection of iron in solutions is most based on spectrophotometric methods, such as the ferrozine assay (Rieme et al., 2004) and the 1,10-phenanthroline method (Pepper et al., 2010). Both techniques depend on the formation of a colored iron (II) complex and its absorbance is measured in relation to a set of standard solutions. However, these Spectrophotometric methods can be limited by the presence of strong complexing agents and possibly by other interferences. Moreover, the need of quite expensive scientific instrument, the requirement of the skilled worker and the sample contamination from transfer to the laboratory prohibit the accessibility to the standard method.

Thus, the digital image-based analysis technique was applied to determine the amount of colorimetric product by literatures (Maleki et al., 2004; Goddijn and White, 2006 Lopez-Molinero et al., 2010). The digital image-based analysis is related to the analysis of Red Green Blue (RGB) basic color data obtained from digital images. Images were generated by a digital camera with the use of complementary metal oxide semiconductor (CMOS), where the reflected light from the colored products of a colorimetric test would pass through and be detected by three different filters: red, green, and blue. After scaling and adjustment in order to compensate for variations in the condition of capture, results were obtained as individual RGB values, and the final color composed of the additive data of the three RGB filters (Choodum and Daeid, 2011). The RGB values derived from the capture of digital images of colorimetric tests can be calibrated with the reference material to obtain the concentration of analysts. In literatures, the RGB were measured by many computer programs, such as MATLAB (Goddijn and White, 2006; Lopez-Molinero et al., 2010) Visual Basic version 6.0 (Maleki et al., 2004) and Kylix version 3.0 (Gaiao et al., 2006; Lyra 2009). Those programs are complicated and inconvenient for low computer-killed people. Recently, our colleague succeeded the use of the ubiquitous software, Adobe Photoshop, to simplify the measurement of the RGB intensity (Srithai et al., 2012; Choodum et al., 2012).

The aim of this study is to explore the use of the digital image analysis coupled with the colorimetric test to measure the iron (II) concentration in water. The reaction condition between iron (II) and color reagent, 1-10-phenanthroline, was optimized to obtain the stable complex product at the lowest reagent amount.

2. Experimental

2.1 Apparatus and reagents

1, 10 - phenanthroline monohydrate and ammonium acetate were obtained from Merck (Darmstadt, Germany). The concentrated hydrochloric acid and glacial acetic acid (AR glade) were purchased from Merck KGaA (Darmstadt, Germany), while ferrous sulfate heptahydrate was bought from Sigma- Aldrich Chemie (Steinheim, Germany). The SONY DSC-WX7 digital camera with Exmor R Cmos sensor (16.2 megapixels) was used in all experiments. The UV-Vis spectrophotometer model Phar 300 M with 1.0 cm quartz cell used as the standard equipment was produced by Merck (Darmstadt, Germany).
2.2 Experiment

The standard iron (II) solution was prepared and constant at 2 ppm in all experiments. Three reagents playing an important role in the colorimetric reaction were prepared as followed. The stock of ammonium acetate buffer solution was prepared by dissolving 25 g ammonium acetate in 15 mL distilled water and 75 mL concentrated glacial acetic acid. The stock solution of 1000 ppm phenanthroline was prepared by dissolving 100 mg 1, 10 – phenanthroline monohydrate in a beaker containing 80 mL distilled water and 1 mL concentrated hydrochloric acid before diluting to 100 mL. For hydrochloric acid solution, the concentrated one (37.7%) was directly diluted to the desired concentration.

The colorimetric reaction was performed in the 1.5 mL centrifuge tube placed in the black box. The 3 watts light bulb was set at 1 cm over the box. This laboratory-built equipment was designed to eliminate the influence of the external light on the image color. The effect of 4 variables; reaction times, amounts of phenanthroline, ammonium acetate buffer and hydrochloric solution, on the reaction product stability were studied. Each batch was photographed by 6 replicates. The camera was set at automatic mode: automatic focus, automatic white balance, automatic sensitivity, whereas the ISO speed was set within 100 to 400 and capture in single image mode. Each image with a size of 1.35 MB (3136×2352 pixel) was recorded as a compress file of JPEG (24-bits). All digital images were transferred into the laptop computer in order to determine the RGB intensity by the Adobe Photoshop version 11.0 combined with Microsoft Excel 2007.

Although the RGB intensity could be used to interpret to the concentration of analyst, the absorbance provided more sensitivity as reported in some literatures (Choodum et al., 2011). The color intensity can be directly converted to the absorbance at each concentration using Equation (1):

\[ A_X = -\log\left(\frac{I_X - I_{X_b}}{I_{X_w} - I_{X_b}}\right) \]  

(1)

Where for each color (R, B, G), \( A_X \) is the absorbance of X, \( I_X \) is the intensity of X, \( I_{X_b} \) = 0 (Intensity of Black color), \( I_{X_w} = 255 \) (Intensity of White color).

In the last section of this study, 2.5 and 4.5 ppm iron (II) solution were prepared and measured by the developed system and the standard method using UV-Vis spectrophotometry to compare the detection efficiency.

3. Results and discussion

3.1 Effect of reaction time

One of important factors for the use semi-quantitative method as a field test was the short time. Therefore, in this study the colorimetric reaction time was firstly optimized.

A series of labeled 1.5 mL centrifuge tube were set and each tube was added by 1.0 mL of 2 ppm standard iron (II), 0.2 mL of 0.3 M ammonium acetate buffer and 0.4 mL of 1000 ppm phenanthroline solution. The reaction times were varied in the range of 1 – 30 min. The results were shown in Fig. 1.

![Fig. 1. Effect of reaction time on color stability by using solution containing 1.0 mL of 2 ppm Iron (II) solution, 0.2 mL of 0.3 M ammonium acetate buffer solution and 0.4 mL of 1000 ppm 1,10 phenanthroline solution.](image)
According to Fig. 1, the RGB absorbance was constant after 5 min reaction time. It was attributed to the formation of the stable complex Fe$^{2+}$-phenanthroline. This was agreed with the standard ASTM E394 method mentioned that the complex color was constant within 5 -10 min (ASTM International, 2004).

Compared with others, the blue color provided the highest absorbance. This was because the reaction product was red-orange and its complimentary color was blue (Tyson Robichaud, 2012).

3.2 Effect of 1, 10-phenanthroline solution concentration

Of all reagents added into the reaction, 1,10-phenanthroline was the most important one, because the complex color was formed by the reaction between the iron (II) and this species. Furthermore, it was also the most expensive chemical when compared with other reagents. On this ground, the amount of phenanthroline was studied. Its concentration was varied in the range of 600 – 1000 ppm at 0.4 mL. The results were presented in Fig. 2.

As shown in Fig. 2, the color absorbance in terms of RGB increased with the increasing 1, 10 - phenanthroline concentration. This was due to the amount of complex more generated by phenanthroline reactant. However, when the phenanthroline concentration was more than 900 ppm, the absorbance became constant. This was because the iron (II), responsible for the complex formation, was used up. The increase in phenanthroline over 900 ppm did not affect to the RGB absorbance.

3.3 Effect of ammonium acetate buffer concentration

Since the pH impacts on the form of complex (APHA, AWWA, WOCF, 1989), it is necessary to use the buffer solution to maintain pH constant. In this research, the concentrations of 0.2 mL ammonium acetate buffer solution were changed in the range of 0.15 -0.30 M. The results were shown in Fig. 3.

As presented in Fig. 3, the highest RGB absorbance was obtained at 0.30 M of 0.2 mL ammonium acetate buffer. This indicated that this concentration was enough to maintain pH constant and made the reaction complex become stable.

3.4 Effect of hydrochloric acid concentration

Like the buffer solution, the acid was applied to the reaction in order to adjust the pH suitable for the product formation. Therefore,
the number of hydrochloric acids was studied, in the range of 0 - 0.4 M. The experimental data could be found in Fig. 4.

![Fig. 4. Effect of different concentrations of 0.2 mL hydrochloric acid solution at 0.2 mL of 0.25 M ammonium acetate buffer solution, 0.4 mL of 900 ppm 1,10-phenanthroline solution and 1.0 mL of 2 ppm Iron (II) solution and 5 min reaction time.](image)

According to Fig. 4, it was revealed that the RGB absorbance was affected by the added hydrochloric acid in the range of study. This might be caused by the pH appropriate for the color reaction. The pH of reaction tube was found at 3.25. It was corresponding to those pH range of 3.2 - 3.3 mentioned in the standard method (Tarafder & Thakur, 2012). Noticed that there was some extent of hydrochloric acid was added into the iron (II) stock solution (in the reagent preparation step) that is why the reaction was in the acidic condition.

3.5 Comparison with the standard method

To determine the detection efficiency, the ATM E394 standard method was applied. The iron (II) solutions of 2.5 ppm and 4.5 ppm were prepared and then measured by both developed system and UV-Vis spectrometry. The results are shown in Table 1.

The data in Table 1 presented the very high accuracy of the developed method. Only 1.60% and 0.67% difference from the prepared concentrations was found by the digital image analysis technique, which was comparable with 0.80% and 0.22% difference obtained by the UV-Vis spectrophotometer standard method.

<table>
<thead>
<tr>
<th>Known Concentration (ppm)</th>
<th>Measured Concentration (ppm)</th>
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<tbody>
<tr>
<td>UV-Vis Spectrometry</td>
<td>Digital Image Analysis</td>
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<tr>
<td>2.50</td>
<td>2.52 ± 0.02</td>
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<tr>
<td>4.50</td>
<td>4.51 ± 0.03</td>
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<tr>
<td>2.46 ± 0.03</td>
<td>2.46 ± 0.03</td>
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<tr>
<td>4.51 ± 0.03</td>
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4. Conclusions

The digital image analysis coupled with the colorimetry was applied to measure the iron (II) concentration in water. The optimization of color reaction was determined at 5 min reaction time, 900 ppm 1, 10-phenanthroline solution, 0.25 M ammonium acetate buffer solution and pH 3.25 without acid adding. This developed method showed the potential to use as the field test with the high accuracy of 0.67 – 1.67% difference from true concentration, in addition to the advantages of low volume reagent, portable device and fast analysis.

5. References


