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Simultaneous Determination of Cr (III) and Cr (VI) Using Spectrophotometric HPLC and EDTA Chelation

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ABSTRACT

A simultaneous determination method for trivalent and hexavalent chromium Cr(III) and Cr(VI) using HPLC with spectrophotometric detection was investigated. By reacting Cr(III) with EDTA, a chelate compound with high absorbance at 410 and 575 nm was generated, allowing simultaneous analysis with dichromate ions (the main form of Cr(VI)) by HPLC. By examining various conditions, the good separation was achieved by changing the wavelength using a reversed phase column, photodiode array detector, and acid eluent. The calibration curve showed good linearity in the range 0.01–10 mM (R^2 = 0.9996–1.000), while detection limits (S/N = 3) for both Cr(III)–EDTA and dichromate were about 0.1 μM when 100 μL of sample was injected. When the chelate and dichromate were added to mineral water, good recovery rates were obtained (88.6%–109.0%). The oxidation of Cr(III) added to tap water by residual chlorine to give Cr(VI) was also monitored.

INTRODUCTION

Chromium (Cr) has both toxic and beneficial effects[1–4]. In mammals, trivalent Cr (Cr(III)) is considered essential for glucose metabolism[1,2], while hexavalent Cr (Cr(VI)) shows toxicity, such as inducing mutation and carcinogenicity[3,4]. In particular, Cr(VI) is of great importance in society[5]. Furthermore, as Cr(III) obtained from the diet is usually sufficient to maintain physiological activity, Cr(III) deficiency rarely occurs. In contrast, Cr(VI) is highly toxic and can be detected in environmental water (such as groundwater) and soil, with permissible levels established[5]. However, Cr(III) is easily oxidized under alkaline conditions to produce Cr(VI). For this reason, attention must be paid not only to wastewater from factories using Cr(VI), but also environmental water containing Cr(III) resulting from contact with concrete and tap water mixed with Cr(III)[6]. Although Cr is mainly present in these two forms in environmental samples and biological samples[4,7,8], determining the concentration of each form, rather than the total amount of Cr, is important owing to their different actions[9].

Inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectrometry (AAS) are commonly used to measure Cr. These methods have high sensitivity, but can only measure the total amount of Cr. A quantitative method for determining each form, which quantitatively measures Cr(III) using colorimetry, followed by reduction, with the resulting increase taken as the Cr(VI) measurement, has been reported, although the sensitivity is slightly inferior[10]. An alternative method, in which only Cr(VI) is subjected to colorimetric quantification followed by oxidation, with the resulting increase taken as the Cr(III) measurement, has also been reported[5,11,12]. However, these methods have complications when the Cr(III) and Cr(VI) concentration ratios are extremely different, giving large measurement errors that prevent accurate measurement. To solve this problem, a method for measuring Cr(III) and Cr(VI) simultaneously using a single measurement system is desirable.

In this study, a simultaneous analytical method for Cr(III) and Cr(VI) determination using HPLC was investigated that takes advantage of the fact that only Cr(III) forms a chelate with EDTA, resulting in a large molecular anion. Basic studies were also performed to determine whether the pretreatment operation was simplified and the storage stability improved by EDTA pretreatment and preservation of the sample.

MATERIALS AND METHODS

Reagents

Potassium chromate and potassium dichromate were obtained from Wako Pure Chemical Industries (Osaka). Chromium (III) nitrate was obtained from Nacalai Tesque (Tokyo). EDTA salts of 4H, 2Na, 3Na, 4Na, and Cu(II), Fe(III), Mn(II), Zn(II) complexes were used (all obtained from Dojindo Laboratories, Kumamoto). Magnesium chloride (Wako Pure Chemical Industries) was used to investigate the influence of coexisting substances. Special reagent-grade sulfuric acid, used as a HPLC eluent, was obtained from Wako Pure Chemical Industries.

The water used for reagent preparation and HPLC analysis was purified to a specific resistance value of ≥18 MΩ·cm using an Elix 3/Advantage water purification system (Merck Millipore, Billerica, MA).

Apparatus

A Prominence system manufactured by Shimadzu Corporation was used as the HPLC apparatus. This system was equipped with an LC-20AT liquid pump (used at 1 mL/min), a DGU-20A3 degasser, a CTO-20AC column oven (40 °C), an SPD-M20A PDA detector, and a SIL-10AF autosampler, and was controlled by a CBM-20A system controller and an LC Solution Multi-PDA workstation. A Senshu Pak PEGASIL ODS SP 100 AQ column (4.6 φ × 250 mm) was used as the analysis column in reversed phase mode. A U-5100 spectrophotometer (Hitachi, Ibaraki) was used for spectrum and absorbance measurements in the preliminary test.

HPLC conditions for chromium compound measurements

For HPLC analysis, the retention behavior on the analytical column must be varied and time resolvable. In the preliminary test, the mutual separation was confirmed by absorbance detection using an anion exchange column (Senshu Pak SAX-1251-N, 4.6 φ × 250 mm) and a reversed phase column (Senshu Pak PEGASIL ODS SP 100, 4.6 φ × 250 mm). The reversed phase column afforded an excellent peak shape and mutual separation, and the retention time was easily changed by varying the organic solvent content. Therefore, the reversed phase column was selected and examined in more detail under further detection conditions. The

quantity for analysis was also investigated using this method.

Pre-addition of chelate compounds to samples for chromium compound measurement

Generally, Cr(III) is known to react slowly with EDTA in aqueous solution through hydration. Therefore, the reaction conditions (such as pH and temperature) for EDTA complex formation were investigated. However, EDTA also forms a chelate with other ions, such as calcium and magnesium. Most EDTA is consumed by reacting with alkaline earth metals present at high concentrations, which might result in an incomplete reaction with Cr(III) ions. Therefore, we focused on both EDTA alone and metal–EDTA complexes, investigating whether Cr(III) chelates could be formed selectively through complex substitution. Furthermore, as valence changes occur in the environment, it is necessary to measure the valence even after sampling. To achieve accurate analysis, both studying the simultaneous measurement and improving the sample stability was important. We also considered adding EDTA compound beforehand as a pretreatment to prevent morphological changes.

RESULTS AND DISCUSSION

Conditions for chromium compound detection

Fig. 1 shows the absorption spectra of Cr(III), Cr(III)–EDTA, chromate, and dichromate. The absorption spectra of chromate and dichromate were similar, but the dichromate absorbance was higher, while the absorption spectra of other substances were mostly different. The Cr(III) absorbance was very small. Therefore, attempting to measure Cr(III) spectrophotometrically gave inferior sensitivity, suggesting that derivatization was essential. From these results, two kinds of Cr(VI) compound seemed to be detected at a detection wavelength of 350 nm, while Cr(III) was detected sensitively at 545 nm if the EDTA chelate was produced quantitatively. The results of the preliminary test using the reversed phase column showed a good peak shape, but weak retention and incomplete separation. When organic solvent was added to the mobile phase, the retention was further weakened and the separation worsened. Therefore, we decided to use the column Senshu Pak PEGASIL ODS SP 100 AQ column (4.6 φ × 250 mm), available in 100% water, to further investigate the eluent for this study. A typical chromatogram is shown in Fig. 2. With sulfuric acid as eluent, the best separation of chromate and dichromate was obtained using a concentration of 0.01 N but was still insufficient for quantitative analysis Fig. 3. However, chromium undergoes a

morphological change with decreasing pH, with chromate transforming into dichromate. This phenomenon has already been reported previously[16], meaning that real liquid samples for metal analysis are mostly stored under acidic conditions. Therefore, the Cr (VI) analysis was only performed for the dichromate form.

Quantitative analysis

Figure 4 shows an example of calibration curves of Cr(III)–EDTA and dichromate. When 10 μL of sample was injected, good linearity was obtained in the concentration range of 0.01–10 mM for peak height and 0.03–10 mM for peak area (R^2 = 0.9996–1.000). The results from adding Cr(III)–EDTA and dichromate to mineral water are shown in Table 1, with good recovery rates of 88.6%–109.0%. The lower detection limits of Cr(III)–EDTA and dichromate were about 5.2 ppb for Cr when calculated with an S/N ratio ≥ 3 (sample injection volume, 100 μL). The Cr(VI) levels permitted by the Ministry of the Environment, Japan, are 50 ppb in soil eluate or 500 ppb in wastewater (both upper limits). Quantification at such a low level was difficult using this method, but it was considered suitable for qualitative examination of the presence or absence of Cr.

Investigation of chelate formation conditions

The reaction conditions of EDTA and Cr(III) for HPLC analysis were investigated. Using various EDTA compounds, after heating at 80 °C for 10 min, the absorbance was measured at 545 nm, which was the maximum absorption of the complex. When 0.1 M 2NA (EDTA·2Na) was used, the absorbance was highest at the same level as when left overnight at room temperature. The absorbance with changing reaction time was similarly examined at 80 °C, with the highest absorbance obtained using EDTA·2Na, with a reaction time of 10 min found to be sufficient. Further examination of the heating temperature showed that the reaction tended to proceed faster as the temperature increased in the examined range of 40–80 °C. However, due to the limited temperature control capability of the water bath used in this experiment, other temperatures were not examined. The influence of pH was confirmed by adding NaOH to various EDTA samples (salts of 4H, 2Na, 3Na, and 4Na). At pH levels of ≤3 or ≥7, EDTA crystals or Cr(III) hydroxide precipitate was observed, respectively. Furthermore, when 100 times the concentration of Mg was present, a decrease in absorbance, attributed to incomplete Cr(III) complex formation, was observed at pH 4 or lower. Therefore, the optimum pH range was considered to be about 4.5–6.0, with lower pH values among this

range, considered better owing to the reactivity of other heavy metals with EDTA. For example, when EDTA·2Na aqueous solution was used as a reagent, pH 4.5 was obtained, which was appropriate Fig. 5.

From the above results, the quantitative chelate formation was achieved when EDTA·2Na aqueous solution was added to the sample and allowed to stand overnight at room temperature or heated at 80 °C for 10 min.

Sample storage stabilization

At the start of this study, we aimed not only to establish an analytical method, but also a preservation method for the accurate quantification of Cr compounds. Although Cr(III)–EDTA and dichromate can be separated and analyzed using this method, we considered it necessary to determine whether these substances changed chemical form during storage. This was related not only to the preservation method but also to sample preparation. The chelate formation between Cr(III) and EDTA is very slow, as reported previously[13]. Therefore, if possible, adding EDTA to the sample and storing before measurement would be advantageous due to avoiding preprocessing labor.

In this study, the concentrations of dichromate and chelate were checked at 350 nm and 545 nm, respectively, as monitoring wavelengths after reacting at room temperature for one week, based on the premise that the additively of absorbance was satisfied. Cr(III) alone, dichromate alone, chelate alone, the chelate and dichromate mixture, and no addition were used as the compounds contained in the sample, while water, tap water (with 0.5 ppm chlorine), 0.1% ascorbic acid, 0.1% chlorine bleach (containing 0.01% chlorine), and 1% chlorine bleach were used as matrixes. Cr(III) oxidation by chlorine and dichromic acid reduction were observed in the ascorbic acid solution, while Cr(III) was resistant to oxidizing agents when chelated Fig. 6. No change in absorbance at 545 nm was observed, even after storage in sulfuric acid.

Furthermore, Cr compounds other than the chelate were susceptible to morphological changes. For example, at low pH, dichromate was reduced to Cr (III) and the evaluated amount of Cr(VI) was very low. As this reaction was faster than chelation, acidification for sample preservation and prior EDTA addition led to a pH change and morphological change in the current reaction system. To prevent this, a chelating agent that reacts more quickly, but

has a weaker chelate forming ability than EDTA, such as diphenylcarbazide, seems to be needed for masking before quantification. Therefore, it is possible to suppress changes in form that accompany redox[18].

Application to real samples

Cr(III) (0.01 mM) was added to tap water and analyzed over time using the HPLC autoinjector function, and Cr(VI) compounds generated from oxidation by residual chlorine contained were monitored, with the results shown in Fig. 7. In this trial experiment, the detection concentration was outside the quantitation range previously obtained. As the sample used was initially almost neutral and had been acidified for only a short time when subjected to analysis, it was possible to measure both chromate and dichromate. However, when the retention time was considered, the only compound directly detected was dichromate. This simple observation of Cr(III) changing to Cr(VI) in the presence of chlorine was one possible outcome.

Although the Cr(VI) form in the above experiment is unknown, Cr(VI) compound detection in the actual sample was possible. The remaining challenge in this study was the weak retention of the substances proposed for measurement in the column. Simultaneous analysis or separation from other coexisting substances might be incomplete for complex matrixes or extreme concentration ratios. To improve this, connecting multiple columns in series, using longer columns, or using both ion exchange and reversed phase columns in series can be considered[19,20]. In the future, we aim to attempt these methods to achieve more complete separation and apply them to other samples.

CONCLUSIONS

To quantify Cr(III) and Cr(VI) compounds simultaneously, a reversed-phase HPLC method was studied. Unfortunately, separating chromate and dichromate was difficult regarding measurement conditions and sample preservation. However, this method provided superior quantitation and a lower detection limit, allowing detection even at concentrations below the environmental standard value for wastewater.

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Table 1. Recovery of Cr(III) and Cr(VI) compounds from mineral water.

Sample composition (Cr(III):Cr(VI))	Cr(III)				Cr(VI)			
	P.H.		P.A.		P.H.		P.A.	
	Recovery (%)	The CV (%)	Recovery	CV	Recovery	CV	Recovery	CV
0.01 mM 1:9 mix	105.22	4.44	109.02	7.26	104.93	2.00	104.72	0.72
0.01 mM 9:1 mix	102.48	1.87	104.24	3.00	91.76	1.09	90.23	1.49
0.01 mM 5:5 mix	102.04	1.74	101.61	2.28	98.51	2.24	97.09	1.86
0.1 mM 1:9 mix	101.79	0.38	99.37	1.48	100.64	0.50	100.40	0.19
0.1 mM 9:1 mix	100.18	0.45	100.17	0.54	88.56	3.84	90.15	9.73
0.1 mM 5:5 mix	101.76	0.79	100.76	0.76	100.21	0.96	102.81	1.25

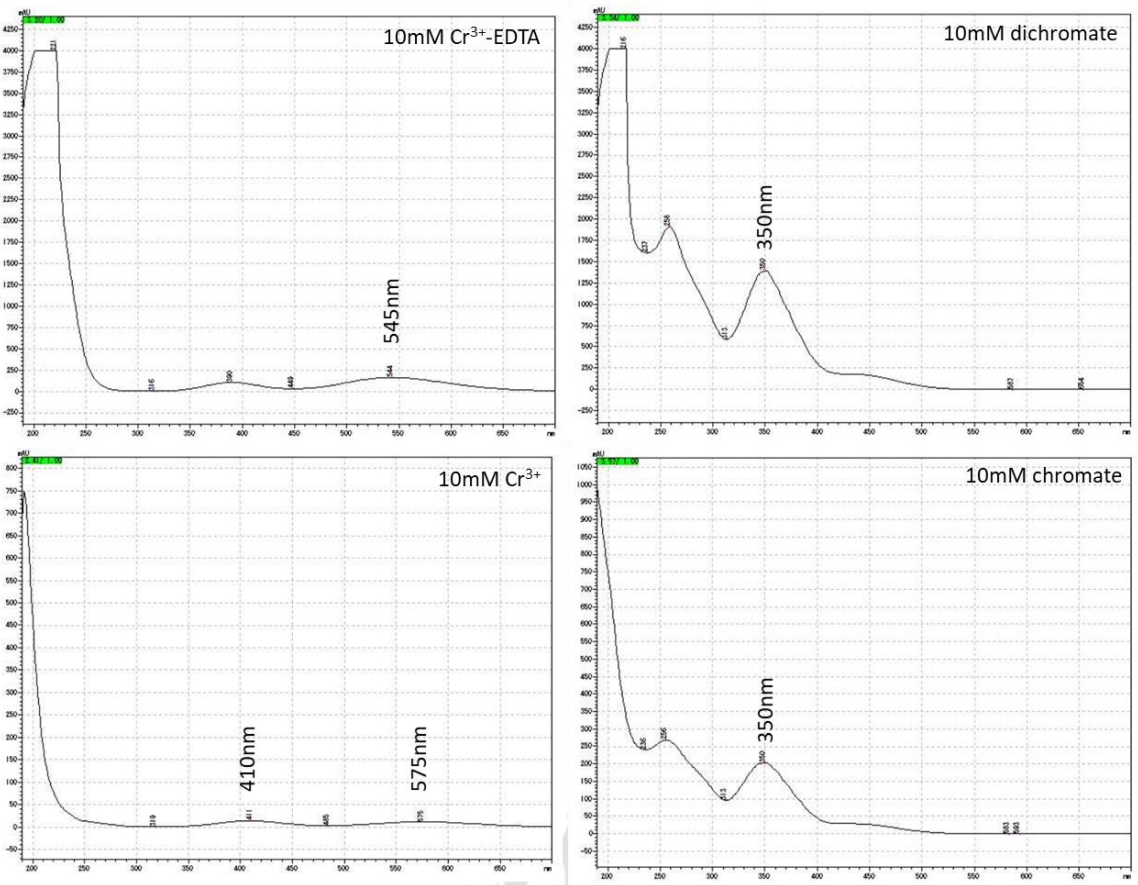

Fig. 1. Absorption spectra of Cr(III), Cr(III)–EDTA, chromate, and dichromate.

Measured using a HPLC photodiode array detector.

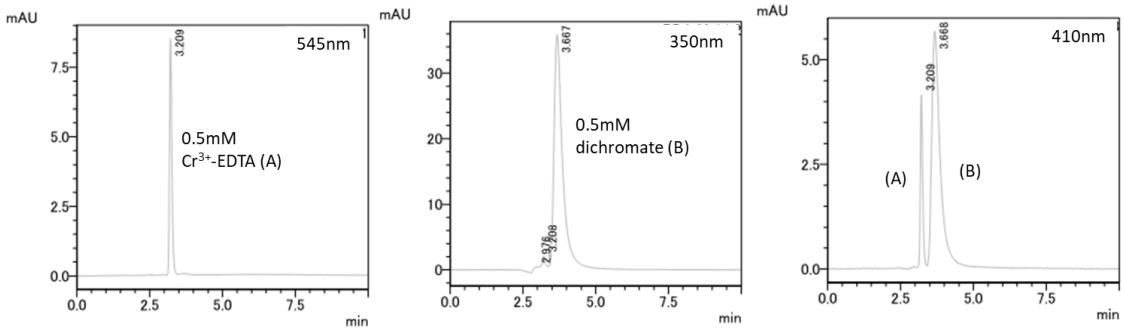

Fig. 2. Typical chromatograms differing only in detection wavelength.

Injection volume, 10 µL; eluent, 0.01 N sulfuric acid; flow rate, 1 mL/min; analytical column, Senshu Pak PEGASIL ODS SP100AQ (4.6 × 250 mm); column temperature, 40 °C.

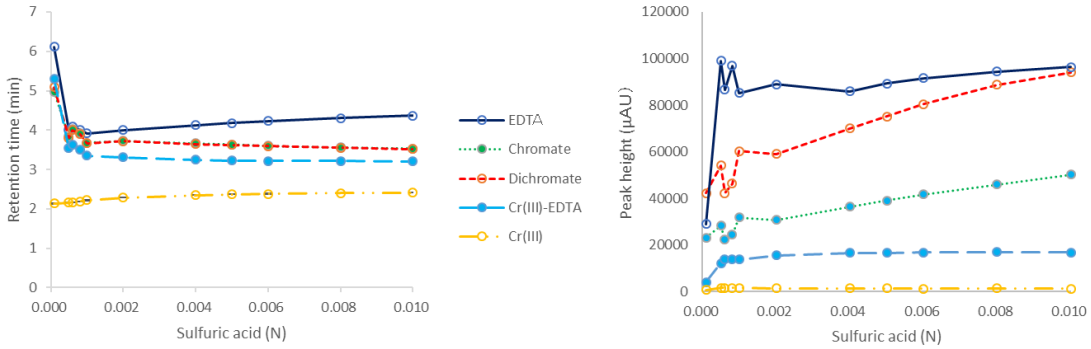

Fig. 3. Effect of sulfuric acid concentration on retention time and peak height.

Detection performed at optimal wavelengths. Injection volume: 10 μL.

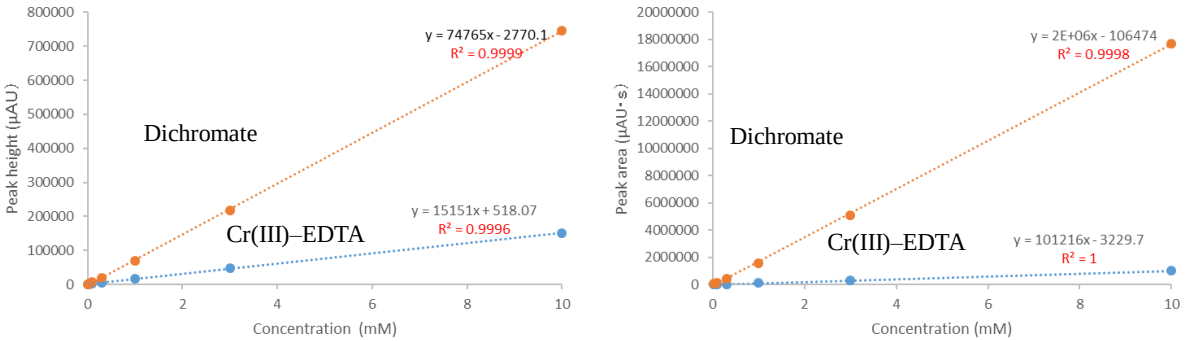

Fig. 4 Calibration curves.

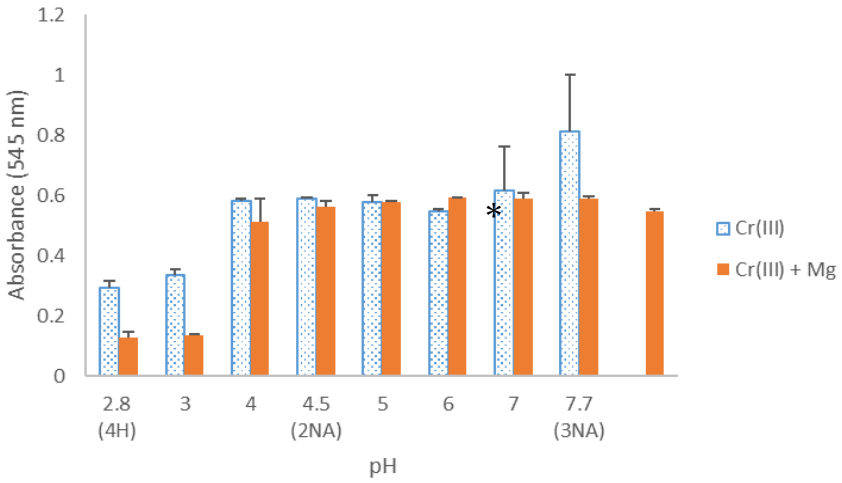

Fig. 5. The difference in reactivity at various pH levels in the presence of a high magnesium concentration.

Measured using a spectrophotometer. Indicated pH is that of the buffer adjusted by adding NaOH to the EDTA compound. Considered to react quantitatively when the absorbance was about 0.6. *Precipitation occurred.

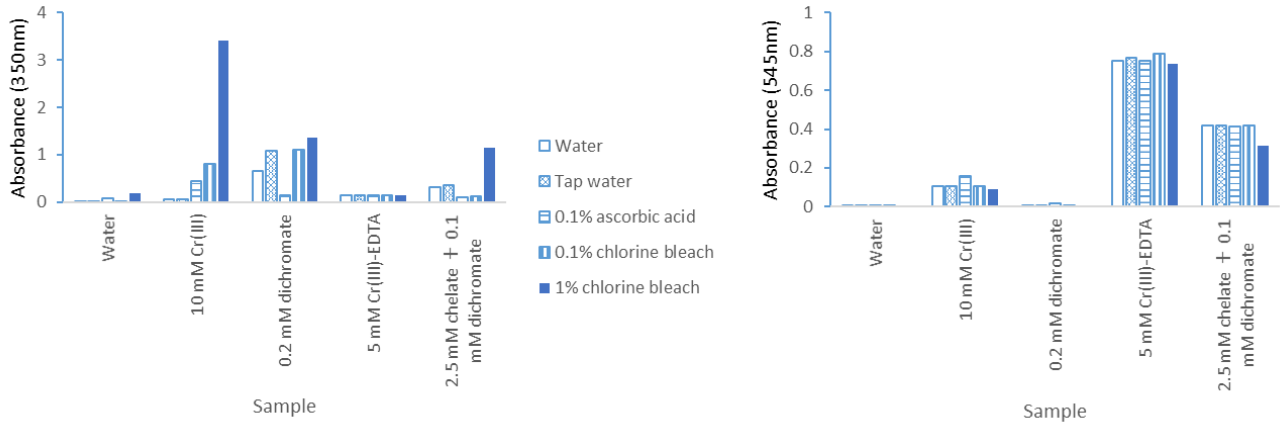

Fig. 6. Influence of matrix when stored for one week.

Measured using a spectrophotometer. Absorbance at 350 and 545 nm mainly represents the dichromate and Cr(III)–EDTA chelate concentrations present, respectively.