

Optimal Sample Preservation and Analysis of Cr(VI) in Drinking Water Samples by High Resolution Ion Chromatography Followed by Post Column Reaction and UV/Vis Detection*

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ABSTRACT

A recent study by the Environmental Working Group reported the detection of hexavalent chromium (Cr(VI)) in tap water at 31 out of 35 states investigated in the United States. Even though Cr(III) is an essential element for human diet, Cr(VI) is a potential carcinogen. Previous work has clearly identified a linear trend of increasing risk of lung cancer mortality with increasing cumulative exposure to water soluble Cr(VI). Regardless, Cr(VI) is still not regulated or monitored in drinking water in the US. There is an existing method (EPA 218.6) for the analysis of Cr(VI), however, this analytical method does not address detailed sample preservation techniques and optimization process to achieve lowest detection limit possible. In this study, five buffer solutions with pH of 9 and above were compared to determine the most suitable buffer to preserve Cr(VI) in drinking water samples for an extended period of time. Results showed that the five buffers responded very differently to Cr(VI)-fortified drinking water. The best preserving reagent was found to be Ammonium Hydroxide + Ammonium Sulfate (pH 9.2) and Sodium Carbonate + Sodium Bicarbonate+ Ammonium Sulfate (pH 9.7), whereas a buffer solution with Sodium Hydroxide + Sodium Carbonate (pH 11.5+) resulted in a poor chromatographic resolution. A controlled study with a fortified Cr(III) at 1 ppb was also conducted to ensure no false positive detection of Cr(VI) due to the potential oxidation of Cr(III) during sample storage. The optimal preserving reagent identified from this study was compatible with the existing EPA method 218.6 using ion chromatography followed by post column reaction, with a method quantitation limit of 0.020 ppb and matrix spike recovery of $\pm 10\%$.

Keywords: Hexavalent Chromium; Ion Chromatography; USEPA Method; Sample Preservation

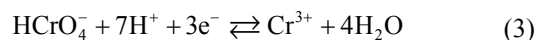
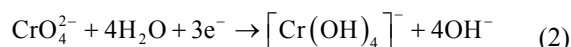
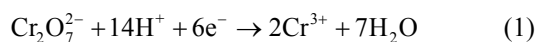
1. Introduction

Chromium (Cr) exists in oxidation states varying from -2 to $+6$ [1], but exists predominantly in the environment in two stable forms, *i.e.*, trivalent {Cr(III)} and hexavalent {Cr(VI)} chromium. Cr(III) is a known essential element for both animals and humans, whereas Cr(VI) in either oxyanionic forms as chromate (CrO_4^{2-}) or dichromate ($\text{Cr}_2\text{O}_7^{2-}$) and bichromate (HCrO_4^-), is a known toxin

and carcinogen. It has been reported that the yellowish coloration of water is due mainly to the presence of monomeric specie of Cr(VI) at concentrations greater than 1.0 mg/L while the orange coloration is due to the presence of high levels of the dichromate, $\text{Cr}_2\text{O}_7^{2-}$. Chromium contamination in the environment can occur through leakages, improper waste disposal or poor storage [2,3].

Conversion between these two major forms of chromium can occur at their respective environmental conditions either in the ambient water or during sample storage period. Cr(VI) is a strong oxidant, which can be easily reduced to Cr(III). The equations below show the reduction of different species of Cr(VI) in the presence of a reducing agent (an electron donor):

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The conditions favorable for the reduction of Cr(VI) to Cr(III) include acidic pH [4], presence of low dissolved oxygen concentration, organic matter [5] or humic substances [6] and the presence of reducing agents such as Fe(II) [5] and sulfide [7]. The mechanism and chemical pathway for such redox reactions has been extensively studied [8]. The conversion of Cr(III) to Cr(VI) has also been investigated under the presence of high dissolved oxygen [7], presence of oxidizing agent such as manganese oxide [9] under acidic or slightly alkaline conditions [0].

It is therefore essential that a method for the reliable analysis of Cr(VI) in environmental samples should ensure the integrity of the Cr species to be preserved and such a preserving reagent will be compatible with the existing method. Currently, the US EPA method 218.6 is the commonly used regulatory method for the analysis of Cr(VI) in surface and drinking water samples. Unfortunately this method does not specify details regarding the preservation of Cr(VI) and Cr(III) originally present in samples, thereby having the potential to report Cr(VI) either false positive or false negative due respectively to the oxidation of Cr(III) and reduction of Cr(VI). This study was initiated in part due to the discovery of Cr(VI) by the Environmental Working Group (EWG) (December 2010) [4] that 31 out of 35 investigated states in the U.S. detected the presence of Cr(VI) in the drinking water. In certain states, detected Cr(VI) averaged about 300%

above the proposed 0.02 ppb limit by the state of California (June 2011).

Figure 1 shows the basic principles of the ion chromatography method used in this study. Cr(VI) after preservation at pH > 9.5 exists as oxyanion CrO_4^{2-} or $\text{Cr}_2\text{O}_7^{2-}$. This oxyanion was chromatographically separated from common anions in drinking water using high resolution anion exchange column. After separation, chromate ion is then reacted with 1,5-diphenyl Carbazide color reagent in the presence of excess acid to form magenta color complex, which is subsequently subject to the detection by a UV-VIS spectrophotometer at 530 nm wavelength.

2. Experimental

2.1. Apparatus and Reagents

Standard solutions of Cr(VI) and Cr(III) were prepared using DI Water (18 MΩ) and pure reagent grade compounds. A list of reagents used is shown in **Table 1**.

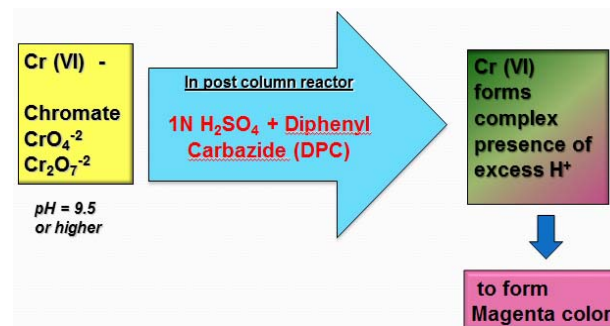


Figure 1. Principle of the ion chromatography followed by post column reaction for the analysis of Cr(VI).

Table 1. Chemicals and reagents.

S/No	Reagent	Chemical Formula	Manufacturer	CAS Number	Percentage Purity (%)
1.	Methanol HPLC grade	CH ₃ OH	Fisher Chemical	67-56-1	99.5
2.	1,5-Diphenylcarbazide	C ₁₃ H ₁₄ N ₄ O	Sigma	140-22-7	
3.	Conc. Sulfuric Acid ACS grade	H ₂ SO ₄	Mallinckrodt	7664-93-9	98
4.	Potassium Dichromate	K ₂ Cr ₂ O ₇	Sigma	7778-50-9	99.9
5.	Sodium Hydroxide	NaOH	ACROS Organic	13-10-73-2	97.7
7.	Sodium Carbonate	Na ₂ CO ₃	Sigma Aldrich	497-19-8	99.5
8.	Sodium Bicarbonate	NaHCO ₃	Sigma	144-55-8	99.5
11.	Ammonium Hydroxide	NH ₄ OH	Sigma-Aldrich	320145	27-29
12.	Ammonium Sulfate	(NH ₄) ₂ SO ₄	Aldrich	204501	99.9
14.	Sodium Tetraborate Decahydrate	Na ₂ B ₄ O ₇ ·10H ₂ O	Sigma-Aldrich	1303-96-4	99.5 - 101.5
15.	Chromium(III) Nitrate	Cr(NO ₃) ₃ ·9H ₂ O	Fisher	7789-02-8	99.9

2.2. Instrumentation

The ion chromatography system employed in this study was Metrohm IC system (model # 850 Professional IC AnCat version) consisting of an auto-sampler (model # 858 Professional AS), with 6 port injection valve (2000 μL injection loop), dual metal-free pumps, a column oven, post column reactor (PCR Box) and a Metrohm UV detector (model # 887). Major instrumental conditions are given in **Table 2**.

A strict QA/QC protocol was maintained for the entire experiment as per USEPA method guidelines [10]. Each sequence run contained DI water blank to demonstrate lack of carryover due to the instrument or any of its components. A 0.1ppb standard containing the analyte of interest was measured to show the accuracy of the current calibration. The percent recovery was within 10% (**Figure 2**).

2.3. Experimental Setup

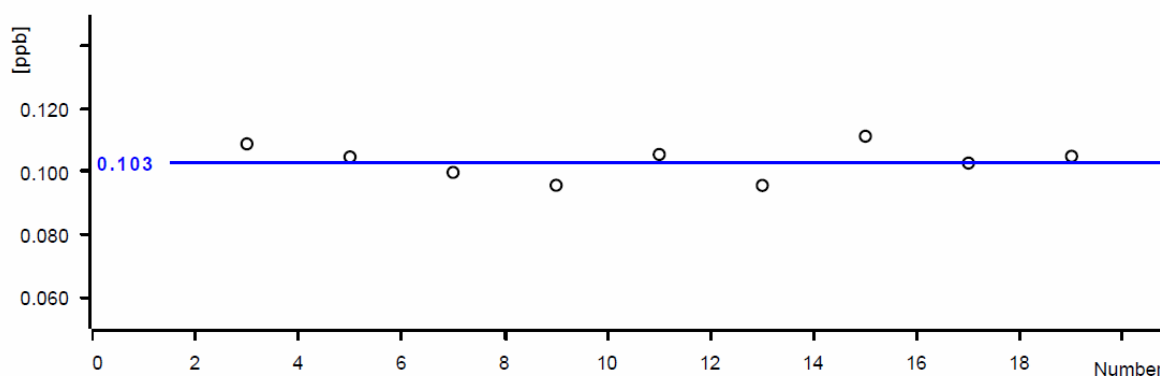
a) Preparation of Mobile Phase: The IC mobile phase was prepared by adding 1.3568 g sodium carbonate, 0.336 g sodium bicarbonate, and 0.25 g ammonium sulfate in 1 L volumetric flask. Dilution was made by using 18 mega-ohm DI water produced by a series of activated carbon, cationic/anionic exchange column, and finally 0.22 μm filter. Alternatively, pre-made concentrated carbonate/bicarbonate can be used.

b) Preparation of Post Column Reagent: The color reagent was prepared by first dissolving 0.5 g 1,5-diphenyl Carbazide (DPC) into 50 mL HPLC grade methanol, then adding this dissolved DPC into approximately 450 mL of 18-mega-ohm DI water in a 1 L volumetric flask. This was followed by gently adding 28 mL concentrated H_2SO_4 and finally diluted with DI water to 1 L. This light sensitive solution was kept in dark prior to use.

Table 2. Method parameters for the analysis of Cr(VI) using IC with post column reaction.

Method Parameters	Analytical Conditions
IC Column	Metrosep ASUPP5-150
Oven Temperature ($^{\circ}\text{C}$)	45
Mobile Phase	12.8 mmol/L Sodium Carbonate + 4.0 mmol/L Sodium Bicarbonate + 2.5 mM Ammonium Sulfate
Column Flow Rate	0.7 mL/min
Post Column Reagent (PCR)	2 mmol/L 1,5-Diphenyl Carbazide + 1 N H_2SO_4
PCR Flow rate	0.25 mL/min
UV Wavelength	530 nm
Injection Volume	2000 μL

chrom-6



Statistics

Mean value:	0.103 ppb	Absolute standard deviation:	0.005 ppb
Minimum:	0.096 ppb	Relative standard deviation:	4.998 %
Maximum:	0.111 ppb	Number of determinations:	10

Figure 2. CCV statistics for 200+ injections.

c) Preparation of sample preservation buffer: Five buffer solutions were tested for their potential as the preserving reagent for Cr(VI). Buffer A was selected because it is suggested by USEPA method 3060A and 1636, whereas buffer C was used in USEPA method 218.6 [11, 12]. As noted in **Table 3**, all buffers have pH of higher than 9.0.

3. Results and Discussion

3.1. Initial Calibration and Quality Control for Entire Experiment

Multi-point calibration was established for analytical range as per **Table 4**.

3.2. Sample Chromatography and Calibration Curve (Figures 3 and 4)

Table 3. Preparation of buffer solutions for the preservation of Cr(VI).

Buffer	Chemical Composition	Initial measured pH
A	2 mM Na ₂ CO ₃ + 10 mM NaOH in 1000 mL	12.0
B	1.25 mM Na ₂ CO ₃ + 1.25 mM NaHCO ₃ in 1000 mL	10.2
C	0.4 g (NH ₄) ₂ SO ₄ + 0.65 mL NH ₄ OH in 1000 mL	10.0
D	1.25 mM Na ₂ CO ₃ + 1.25 mM NaHCO ₃ + 0.15 g/L (NH ₄) ₂ SO ₄ in 1000 mL	9.25
E	5 mM NaHCO ₃ + 10 mM Na Tetra Borate in 1000 mL	9.10

Table 4. Calibration standards.

Calibration Level	Chromium(VI), parts per billion (ppb)
Level 1	0.025
Level 2	0.050
Level 3	0.100
Level 4	0.250
Level 5	0.500
Level 6	1.000
Level 7	2.000
Level 8	5.000

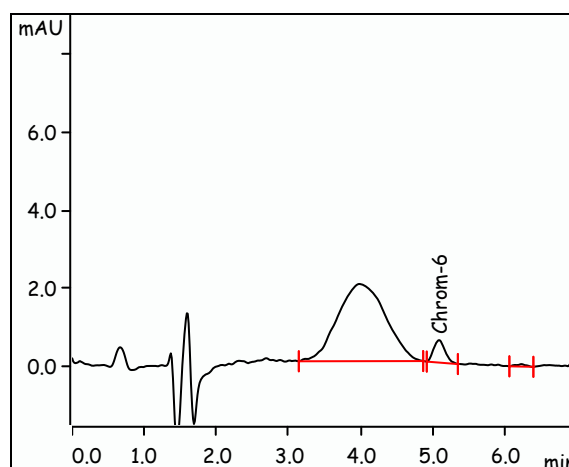


Figure 3. Cr(VI) at 0.025 ppb.

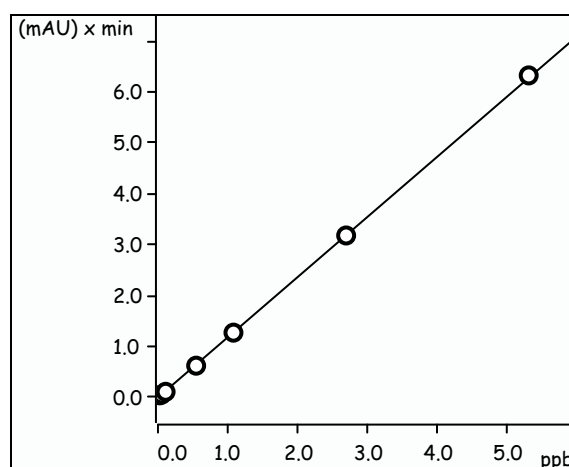


Figure 4. Calibration curve.

3.3. Method Detection Limit (MDL) Study [10]

A method detection limit (MDL) is the minimum concentration of a specified analyte that can be detected and quantified with a 99% confidence level. The MDL was determined by injecting 7 replicates of known concentration near the expected limit of detection. The standard deviation is determined from the results and multiplied by the t value. The t value for 7 replicates is 3.14 at a 99% confidence level. An MDL study was performed for Chromium(VI) over three days. **Table 5** demonstrates the data for the study.

a) Minimum Reporting Limit (MRL) study (new QC parameter for USEPA Chromium (VI) method Analyze seven replicate at or below the proposed MRL concentration. Calculate the mean (Mean) and standard deviation for these replicates. Determine the Half Range for the Prediction Interval of Results (HR_{PIR}) using the equation

$$HR_{PIR} = 3.963S$$

where S is the standard deviation and 3.963 is a constant value for seven replicates.

Confirm that the Upper and Lower limits for the Prediction Interval of Results ($PIR = \text{Mean} \pm HR_{PIR}$) meet the upper and lower recovery limits as shown below.

The Upper PIR Limit must be ≤ 150 percent recovery.

$$\frac{\text{Mean} + HR_{PIR}}{\text{Fortified Concentration}} \times 100 \leq 150\%$$

The Lower PIR Limit must be ≥ 50 percent recovery.

$$\frac{\text{Mean} - HR_{PIR}}{\text{Fortified Concentration}} \times 100 \geq 50\%$$

Table 6 demonstrates analyzed data for MRL study.

b) Continuing Calibration Verification (CCV) for the entire experiment.

CCV of lower range of calibration (0.1 parts per billion) was analyzed every 10 samples. Overall average recovery is 103%.

Table 5. Method detection limit (MDL) study.

	Chromium(VI), (ppb)
MDL-1 (Day 1)	0.064
MDL-2 (Day 1)	0.074
MDL-3 (Day 2)	0.073
MDL-4 (Day 2)	0.058
MDL-5 (Day 2)	0.063
MDL-6 (Day 3)	0.062
MDL-7 (Day 3)	0.061
Average	0.065
Standard Dev	0.006
Calculated MDL	0.019

Table 6. MRL study data.

	Chromium(VI), ppb
analysis-1	0.018
analysis-2	0.018
analysis-3	0.019
analysis-4	0.02
analysis-5	0.018
analysis-6	0.022
analysis-7	0.018
Mean	0.0190
std.Dev	0.0015
HP_{PIR}	0.0061
True Concentration	0.020
Upper HP _{PIR}	125.2679
Lower HP _{PIR}	64.73208

4. Sample Preservation Buffers Data Discussion

1 Liter of each buffer solutions was prepared in two separate 1 Liter plastic containers. One bottle was fortified with 1 part per billion Chromium(VI) and other bottle was fortified with 1 part per billion each of Chromium(III) and Chromium(VI). Every day for consecutive 21 days this solution was analyzed for Chromium (VI) stability. This solution was preserved at 4 deg.C in refrigerator.

4.1. Buffer—A (2 mM Na₂CO₃ + 10 mM NaOH)

Buffer A (**Figure 5**) data demonstrated that even at Day 1 there is almost 52% oxidation of Chromium(III) into Chromium(VI) due to high pH of buffer (pH = 12). Progressively, oxidation is increased and on Day 6 it is 100% conversion. Also, please make a note that data from Day 7-21 indicates more than 100% conversion to Chromium(VI). This is due to original Total Chromium (Cr) contamination in Sodium Hydroxide pellets. This total Cr converted to Cr(VI) with favorable pH in the solution.

4.2. Buffer—B (1.25 mM Na₂CO₃ + 1.25 mM NaHCO₃)

Buffer B (**Figure 6**) data demonstrated that even at Day 1 there is almost 59% oxidation of Chromium(III) into Chromium(VI) due to high pH of buffer (pH = 10.5). Progressively, oxidation is increased and on Day 8 it is 92% conversion. Sodium Carbonate/Bicarbonate buffer preserves Chromium(VI) at a stable rate but if native Cr(III) is present in drinking water then it will also convert it to Cr(VI) very quickly.

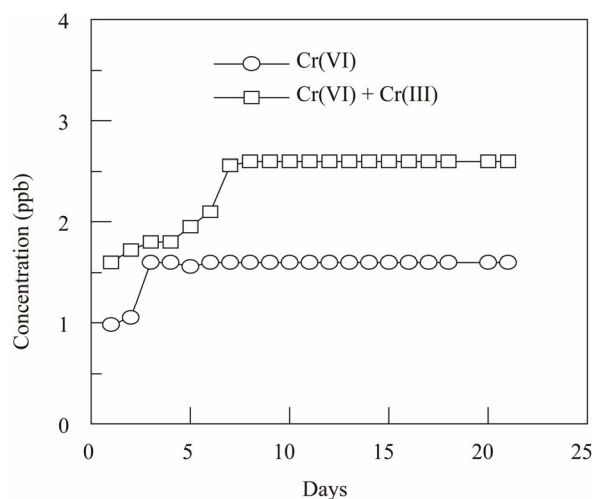


Figure 5. Buffer A data for Cr(III) to Cr(VI) conversion.

4.3. Buffer—C (0.4 g (NH₄)₂SO₄ + 0.65 ml NH₄OH)

Buffer C (Figure 7) data demonstrates that this buffer preserves Cr(VI) very well for at least 21 days.

Buffer—D (1.25 mM Na₂CO₃ + 1.25 mM NaHCO₃ + 0.15g/L (NH₄)₂SO₄)

Buffer D (Figure 8) data demonstrated that this buffer preserves Cr(VI) very well for at least 21 days. It is believed that Ammonium Salt in the buffer system forms Chloramine products due to free chlorine in drinking water samples and hence prevents Cr(III) oxidation to Cr(VI).

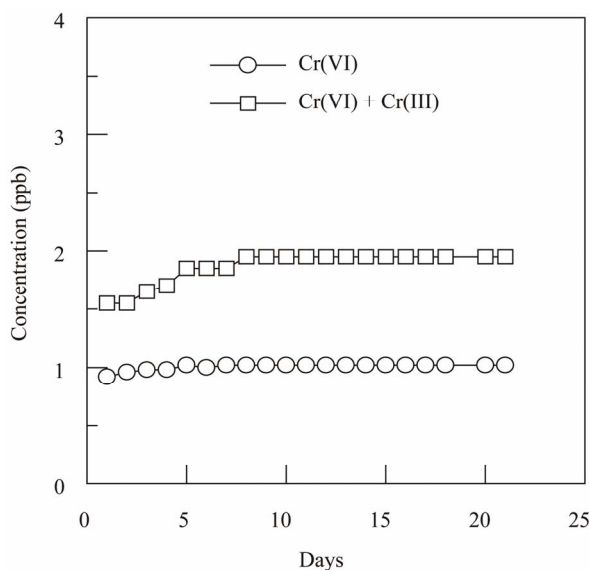


Figure 6. Buffer B data for Cr(III)--> Cr(VI) conversion.

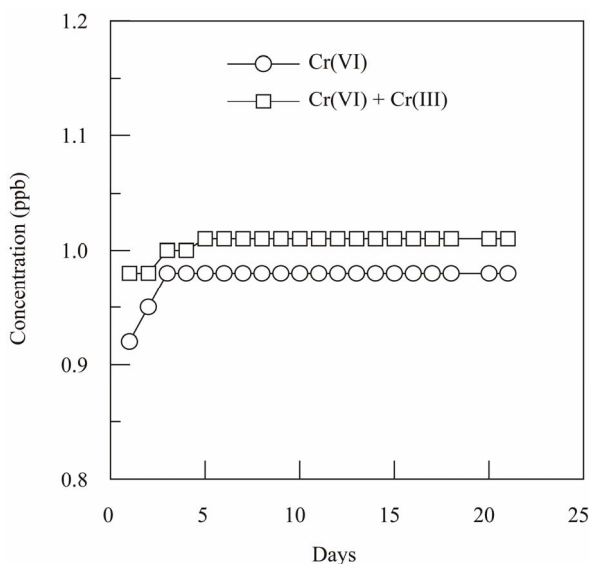


Figure 7. Buffer C data for Cr(III)--> Cr(VI) conversion.

4.4. Buffer—E (5 mM NaHCO₃ + 10 mM Na Tetra Borate)

Borate Buffer originally recommended by CADPH (May 2010) [13]. Buffer E (Figure 9) data demonstrated that this buffer preserves Cr(VI) very well for at least 4 days. Up to 20% Cr(III) oxidizes to Cr(VI). This may be due to native total Chromium present in Tetraborate salt. Analyst had to validate each batch of pure buffer chemicals purchased.

5. Conclusion

This study concludes that there are several choices and flexibility in the selection buffers for the preservation of Cr(VI) in water matrix. We also conclude that buffers containing ammonium salt is favorable for drinking

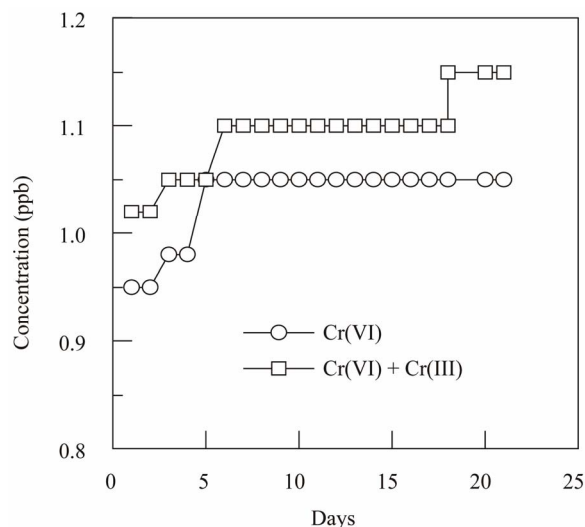


Figure 8. Buffer D data for Cr(III)--> Cr(VI) conversion.

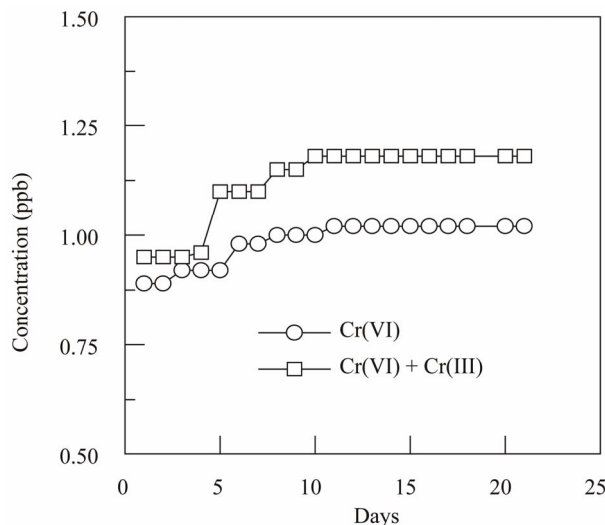


Figure 9. Buffer E data for Cr(III)--> Cr(VI) conversion.

water samples as it contains free chlorine which can serve as a strong oxidizer. Buffer D containing 1.25 mM Na_2CO_3 , 1.25 mM NaHCO_3 , and 0.15 g/L $(\text{NH}_4)_2\text{SO}_4$ added in the solid form of field samples is recommended. This preserving chemical is also compatible with the subsequent analytical method (USEPA method EPA 218.6) [11,14] using IC with post column reaction. Additional results for the evaluating the effects of various oxidizers, reducers, organic material potentially important in drinking water, and pH effect on sample preservation will be reported in a forthcoming paper.

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