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Evaluation of Sample Preparation Procedures for Determination of Cr(VI) in Cr_2O_3 Pigments by Vis Spectrophotometry

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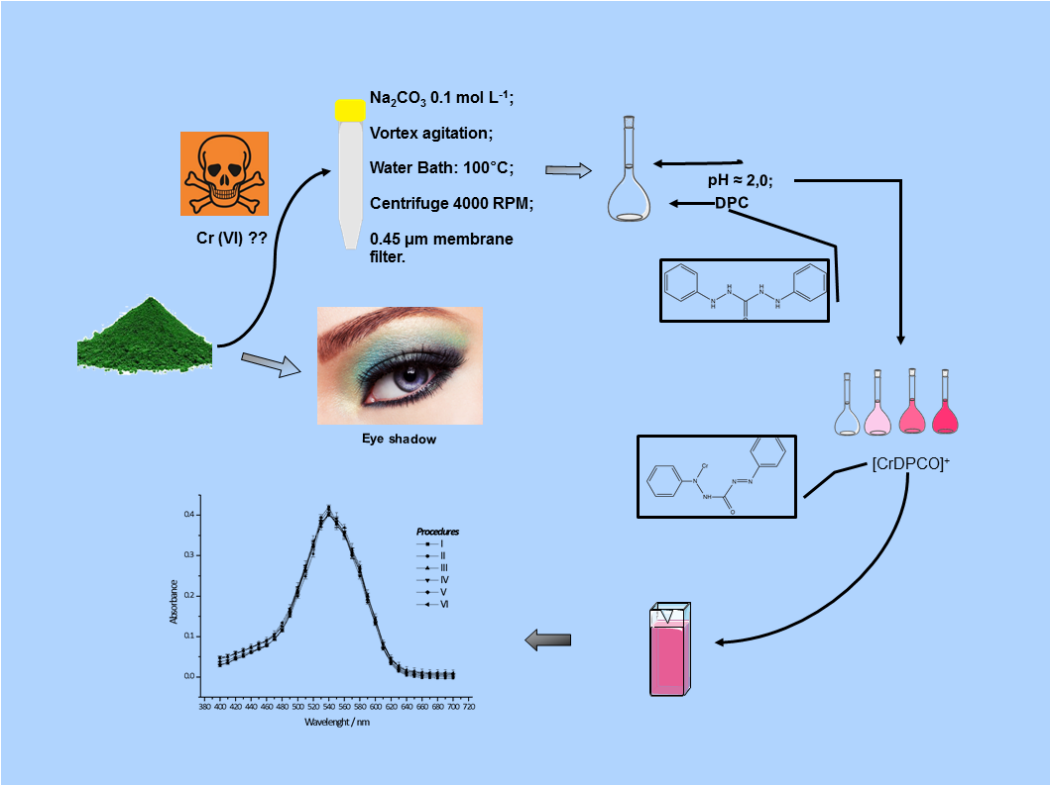
HIGHLIGHTS

- Different preparation procedures were investigated to selectively determine Cr(VI) in Cr_2O_3.
- A robust method was developed for detection of Cr(VI) in Cr_2O_3 pigments for use in cosmetics products.
- Method validation demonstrated accuracy and precision.

Abstract: Six sample preparation procedures were evaluated for selective extraction of Cr(VI) from commercial samples of chromium oxide green (Cr_2O_3) pigments prior to formation of its diphenylcarbazone complex $[CrDPCO]^-$ for determination by visible spectrophotometry: (I) water-soluble chromium; (II) EPA method 3060A without Mg^{2+}; (III) EPA method 3060A with Mg^{2+}; (IV) Na_3PO_4 based extraction; (V) method IRSA16 based on acidic extraction and; (VI) Na_2CO_3 based extraction. Evaluation of the influence of concomitant Cr(III) ions, time and stability of the $[CrDPCO]^-$ complex was investigated. Recoveries of soluble and insoluble Cr(VI) species were 86% and 80%, respectively, using procedure (VI). Direct calibration against aqueous standards prepared in the extraction medium was successful for Cr(VI) in the concentration range 0.05-1.50 μg L^{-1}. Limits of detection and quantitation were 0.3 μg g^{-1} and 1.0 μg g^{-1}, respectively, for 250 mg subsamples/25 mL. Procedure (VI) was applied to the analysis of four commercial samples of Cr_2O_3 pigments, three determined to have Cr(VI) within compliance limits below 1.0 μg g^{-1}, but one at 16.6 ± 0.6 μg g^{-1}, prohibiting use of this pigment in cosmetic formulations. This sample was conveniently employed to evaluate the accuracy of the method. The recommended procedure is simple and accurate and has been adopted by Tecpar's laboratory of Parana Institute of Technology (Curitiba, Brazil).

Keywords: Cr_2O_3; pigments; Cr(VI); Vis spectrophotometry.

GRAPHICAL ABSTRACT



INTRODUCTION

According to the European Community Regulation No 1223/2009 and ANVISA RDC 44/2012, chromium oxide (Cr_2O_3) and chromium hydroxide [$Cr_2O(OH)_4$] pigments are authorized for use as colorants for cosmetic products [1,2]. The European Regulations highlight over 1200 toxic substances whose presence is likewise prohibited. Among them are metals particularly dangerous to human health, including Pb, Cd, As, Ni, Hg and Cr(VI) as chromate ion and its salts [1-4]. The absence of Cr(VI) as chromate ion must be assured in both raw material and finished products as its toxicity is well-known [1,2]. Although Cr(III) is an essential nutrient required for normal energy metabolism, Cr(VI) is highly toxic, a known carcinogen as well as skin allergen active through percutaneous absorption [3-6]. The cosmetic industry requires control of Cr(VI) in pigments (mainly in green $Cr_2O(OH)_4$ and Cr_2O_3) selected for use as well as potential oxidative changes in Cr(III) that may occur during manufacture of final products. A representative example of cosmetic product is eye shadow, to which significant concentrations of $Cr_2O(OH)_4$ and/or Cr_2O_3 are intentionally added as colouring agents [1,3-4]. Spectrophotometry remains a widely used method for detection of Cr(VI) in water, soils, sludges, sediments, and similar solid waste samples based on its complexation with 1,5-diphenylcarbazide (DPC) because of its simplicity, speed and low cost while meeting the required legislated limits of 1 µg g^{-1} [3,7-9]. However, different sample pre-treatment options for solid samples are noted in the literature [9-15]. EPA method 3060A is most often recommended for determination of Cr(VI) in solid environmental matrices and is based on an alkaline extraction [9]. Petrucci and Senofonte [10] modified this approach for application to the determination of Cr(VI) in cosmetic products (eye shadows and face power) by ion chromatography coupled with dynamic reaction cell-inductively coupled plasma-mass spectrometry (IC-DRC-ICP-MS) [10]. Bruzzoniti and coauthors [11] compared three different methods for extraction of Cr(VI): EPA method 3060A, IRSA16 (Istituto di Ricerca Sulle Acque: cromo esavalente) [12] and use of Na_3PO_4 [13] for determination of Cr/Cr(VI), Ni and Co in cosmetic matrices (pearly pigment, black iron oxide, pearly powder and eye shadow samples) using ion chromatography [9,11-13]. Owolabi and coauthors [14] simply leached Cr(VI) and V(V) from medicinal plants with a 0.10 mmol solution of Na_2CO_3 prior to determination by electrothermal atomic absorption spectrometry whereas Mathebula and coauthors [15] employed the same extraction approach for the leaching of Cr(VI) from bread and breakfast cereals for further determination by high resolution continuum source absorption spectrometry (HR-CS AAS) [14,15]. Due to the high risk of contamination by Cr(VI) when

using Cr_2O_3 pigment as a colorant in cosmetic products, it is important to determine the presence of this extremely toxic contaminant. To the best of our knowledge, such a comprehensive comparative study of methodologies in this complex matrix has never been undertaken. Six different sample preparation procedures, all proposed in earlier literature, have been assessed for efficacy, i.e. (I) water-soluble chromium; (II) EPA method 3060A without Mg^{2+}; (III) EPA method 3060A with Mg^{2+}; (IV) Na_3PO_4 based extraction; (V) method IRSA16 based on acidic extraction and; (VI) Na_2CO_3 based extraction. A traditional spectrophotometric technique was used for detection as it is robust, simple, sensitive and permits speciation of Cr(VI) and Cr(III) via a reaction with 1,5-diphenylcarbazide (DPC). Results for the determination of total leachable chromium, i.e., the sum of Cr (III) and Cr (VI), were generated by use of ICP OES, since this technique is rapid, sensitive and suffers few interferences.

MATERIAL AND METHODS

Instrumentation

For determination of leached Cr [sum of Cr(III) and Cr(VI)], emission measurements were undertaken using a VISTA PRO (Varian, Mulgrave, Australia) simultaneous axial view ICP OES spectrometer. Peak height intensities were measured at 267.716 nm. Argon of 99.996% purity was supplied by White Martins (São Paulo, Brazil). The operating conditions for the ICP OES are summarized in Table 1.

Table 1. ICP OES operating parameters.

Parameters	Specifications
Radiofrequency	40 MHz
Radiofrequency power	1.10 kW
Plasma gas flow rate	15.0 L min^{-1}
Auxiliary gas flow rate	1.5 L min^{-1}
Nebulizer pressure	140 kPa
Nebulizer type	OneNeb@ nebulizer (Agilent)
Spray chamber	Standard Cyclonic (Varian)
Replicate read time	3 s
Replicates	3
Torch type	Demountable torch with 1.8 mm I.D. alumina injector tube
Analytical line (Cr)	267.716 nm

A Mettler Toledo model AB204-2 analytical balance (São Paulo, Brazil); Marconi model MA093 temperature controlled water bath (São Paulo, Brazil); Heidolph model multireax vortex agitator (Schwabach, Germany); Unique model USC 1800A (40 khz) ultrasonic bath (São Paulo, Brazil); Eppendorf AG model 5430R centrifuge (Hamburg, Germany); Telastem Peneiras model Granutest stainless steel 35 mesh (500 µm) sieve, (São Paulo, Brazil) and hydrophilic PVDF 0.45 µm membrane filters (Millipore Millex, USA) were used during the preparation of samples. All pH values were determined with a Thermo Orion model 710A+ (New York, USA) potentiometer fitted with a combined glass-calomel electrode. Spectrophotometric measurements were made using a Thermo model BioMate^{TM}3 (New York, USA) in combination with a matched set of standard 10 mm path length quartz sample cells. The wavelength peak of 540 nm was confirmed for use for absorbance measurements.

Reagents and materials

All chemical reagents were of analytical grade, unless otherwise specified. High-purity water (18.2 MΩ cm resistivity) was de-ionized in a Milli-Q system (Bedford, MA, USA). The following reagents, solutions and standards were used: sodium carbonate Na_2CO_3 (99.9 %, Merck, Darmstadt, Germany), sulfuric acid H_2SO_4 (95-97 %, Merck), sodium phosphate tribasic dodecahydrate $Na_3PO_4.12H_2O$ (≥98%, Sigma-Aldrich, St. Louis, USA), magnesium chloride hexahydrate $MgCl_2.6H_2O$ (99.0-102.0 %, Panreac, Barcelona, Spain) and sodium hydroxide NaOH (97.0 %, Reatec, Paraná, Brazil). A pH 7.0 buffer solution consisting of 0.5 mol L^{-1} potassium dihydrogen phosphate KH_2PO_4 (99.5-100.5 %, Merck) and 0.5 mol L^{-1} di-potassium hydrogen phosphate K_2HPO_4 (≥99.0 %, Merck) was prepared by dissolution of 68.04 g KH_2PO_4 and 87.09 g K_2HPO_4 in 700 mL of high-purity water and subsequently diluted to exactly 1000 mL with high-purity water and homogenized. A standard stock solution containing 1000 µg mL^{-1} Cr(III) from AccuStandard (New Haven, USA) was used to prepare external calibration solutions for determination of leached Cr by ICP OES. A standard stock solution containing 50 µg mL^{-1} Cr(VI) was prepared by dissolving 0.14168 g potassium dichromate $K_2Cr_2O_7$ (≥99.8%, Sigma-Aldrich) in exactly 1000 mL high-purity water. Diphenylcarbazide (DPC) solutions were prepared weekly by dissolving 250 mg 1,5-diphenylcarbazide (≥98.0%, Sigma-Aldrich) in 50 mL acetone (99.5%, Êxodo Científica, São Paulo, Brazil). Solutions were stored in a brown bottle. Recovery of water-soluble and insoluble Cr(VI) species after extraction using the different methods was evaluated against both potassium chromate K_2CrO_4 (≥99.0%, Sigma-Aldrich) as well as lead chromate ($PbCrO_4$). The latter was synthesized according to Ferreira and coauthors [16] and Orna [17] by precipitating equimolar quantities of Pb^{2+} and CrO_4^{2-} ions from aqueous solutions of their soluble salts. A typical reaction is:

$$K_2CrO_4 + Pb(NO_3)_2 \longrightarrow 2KNO_3 + PbCrO_4 \quad (1)$$

Four commercial samples of Cr_2O_3 pigment were provided by a local cosmetic company.

Methods

Determination of leached Cr [Cr(VI) + Cr(III)] by ICP OES

Samples prepared following procedures I to VI were made to final volumes of 25 mL without addition of diphenylcarbazide solution, and analyzed. Calibration standards were prepared by serial dilution of the standard stock solution containing 1000 µg mL^{-1} Cr(III) (AccuStandard) in an acidic medium (pH adjusted to 2.0 ± 0.5 by H_2SO_4) to span the concentration range 0.05 - 2.00 µg Cr mL^{-1}. Blank solutions of the same medium were run in parallel to the determinations and their intensities were taken into consideration.

Spectrophotometric determination of Cr(VI)

A 500 µL aliquot of diphenylcarbazide solution was added to prepared 25 mL sample extracts as well as method blanks generated by use of procedures I to VI, mixed and left standing for 5 min for full color development. Calibration standards were prepared by serial dilution of the 50 µg mL^{-1} stock solution of Cr(VI) ($K_2Cr_2O_7$, Sigma-Aldrich) in the same medium as the sample, and spanned the concentration range 0.05-1.50 µg Cr(VI) mL^{-1}. Blank solutions run in parallel to the determinations were used to correct all absorbance measurements [8].

The six different sample preparation procedures for extraction of Cr(VI) from Cr_2O_3 pigment were evaluated. All were applied to one sample in which significant Cr(VI) content was not detected, as described below:

Procedure I - water-soluble chromium

A nominal 250 mg subsample was accurately weighed and placed in a 15 mL volumetric flask to which 10 mL of high-purity water was added and homogenized. The mixture was kept in a water bath for 1h at 80ºC under agitation and subsequently placed in an ultrasonic bath for 15 min at room temperature. Thereafter, the sample was centrifuged at 4000 rpm for 20 min and filtered through a 0.45 µm filter. The pH was adjusted to 2.0 ± 0.5 by addition of 3 mol L^{-1} H_2SO_4 and diluted to a final volume of 25 mL with high-purity water and homogenized [18]. Blank solutions were also prepared in parallel. The determination of Cr(VI) and leached Cr was carried out as described above.

Procedure II – EPA method 3060A without Mg^{2+}

A nominal 250 mg subsample was accurately weighed and placed in a 15 mL volumetric flask to which 10 mL of an alkaline extraction solution (0.28 mol L^{-1} Na_2CO_3 and 0.5 mol L^{-1} NaOH) was added and homogenized. The mixture was kept in a water bath for 1h at 90-95ºC under agitation and subsequently cooled. Thereafter, the sample was centrifuged at 4000 rpm for 20 min and filtered through a 0.45 µm filter. The pH was adjusted to 2.0 ± 0.5 by addition of 3 mol L^{-1} H_2SO_4 and diluted to a final volume of 25 mL with high-purity water and homogenized [9,10]. Blank solutions of the same medium were run in parallel and their absorbance taken into consideration. The determination of Cr(VI) and leached Cr was carried out as described above.

Procedure III – EPA method 3060A with Mg^{2+}

Procedure II was essentially followed with the exception that 16.0 mg of $MgCl_2$ was added to the sample aliquot and blank. The determination of Cr(VI) and leached Cr was carried out as described above [9,10].

Procedure IV – Na_3PO_4 based extraction

A nominal 250 mg subsample was accurately weighed and placed in a 15 mL volumetric flask to which 10 mL of 0.01 mol L^{-1} Na_3PO_4 was added and homogenized. The mixture was kept in a water bath for 5 min at 100 ºC under agitation and subsequently cooled. Thereafter, the sample was centrifuged at 4000 rpm for 20 min but required a double filtration through a 0.45 µm filter to remove particulate matter. The pH was adjusted to 2.0 ± 0.5 by addition of 3 mol L^{-1} H_2SO_4 and diluted to a final volume of 25 mL with high-purity water and homogenized [11,13]. Blank solutions of the same medium were run in parallel and their absorbance taken into consideration. The determination of Cr(VI) and leached Cr was carried out as described above.

Procedure V – IRSA16 based extraction

A nominal 250 mg subsample was accurately weighed and placed in a 15 mL volumetric flask into which 10 mL of an extraction solution consisting of 9.8 mL of high-purity water + 200 µL of a 1:1 mixture H_2SO_4/H_2O. The sample was homogenized and kept under vortex agitation for 10 min. Thereafter, the sample was centrifuged at 4000 rpm for 20 min and filtered through a 0.45 µm filter. The final solution was diluted to 25 mL with high-purity water and homogenized. The pH of the solution was 1.2 ± 0.2 [11,12]. Blank solutions of the same medium were run in parallel and their absorbance taken into consideration. The determination of Cr(VI) and leached Cr was carried out as described above.

Procedure VI – Na_2CO_3 based extraction

A nominal 250 mg subsample was accurately weighed and placed in a 15 mL volumetric flask into which 10 mL of a 0.1 mol L^{-1} solution of Na_2CO_3 was added and homogenized by vortex agitation for 10 min. The mixture was placed in a water bath for 30 min at 100 ºC under agitation and subsequently cooled. Thereafter, the sample was centrifuged at 4000 rpm for 20 min and filtered through a 0.45 µm filter. The pH was adjusted to 2.0 ± 0.5 by addition of 3 mol L^{-1} H_2SO_4 and diluted to a final volume of 25 mL with high-purity water and homogenized [14,15]. Blank solutions of the same medium were run in parallel and their absorbance was taken into consideration. The determination of Cr(VI) and leached Cr was carried out as described above.

RESULTS AND DISCUSSION

Determination of leached Cr by ICP OES

The determination of metals by ICP OES in solutions containing high dissolved solids can affect the nebulization efficiency, may damage or clog conventional concentric nebulizers and requires care due to potential elevated spectral interferences [19]. Use of the OneNeb® (Agilent) in combination with a cyclonic spray chamber and a demountable torch fitted with an alumina injector tube allowed the introduction of solutions containing high dissolved solids into the plasma, compatible with samples prepared according to Procedures II-IV and VI for determination of leached Cr. Three analytical lines were investigated: 205.560 nm, 267.716 nm and 283.563 nm. Through the evaluation of the emission spectra generated by the samples and standards, no significant differences in terms of final results, freedom from both spectral and non-spectral

interferences and adequate sensitivity were noted. Thus, there was no need for matrix matching of the standards, making possible the use of calibration standards prepared in a simple pH 2.0 ± 0.5 acidic medium matching that of the samples. As the 267.716 nm line is commonly used in routine laboratories it was chosen for all subsequent measurements. Figure 1 presents an example of spectral scans acquired during introduction of a prepared sample (Procedure II) and a calibration standard, both of which are noticeably free of any other emission structures. The small structure at higher wavelength does not impact quantitation since peak height intensity at 267.716 nm was used for this purpose.

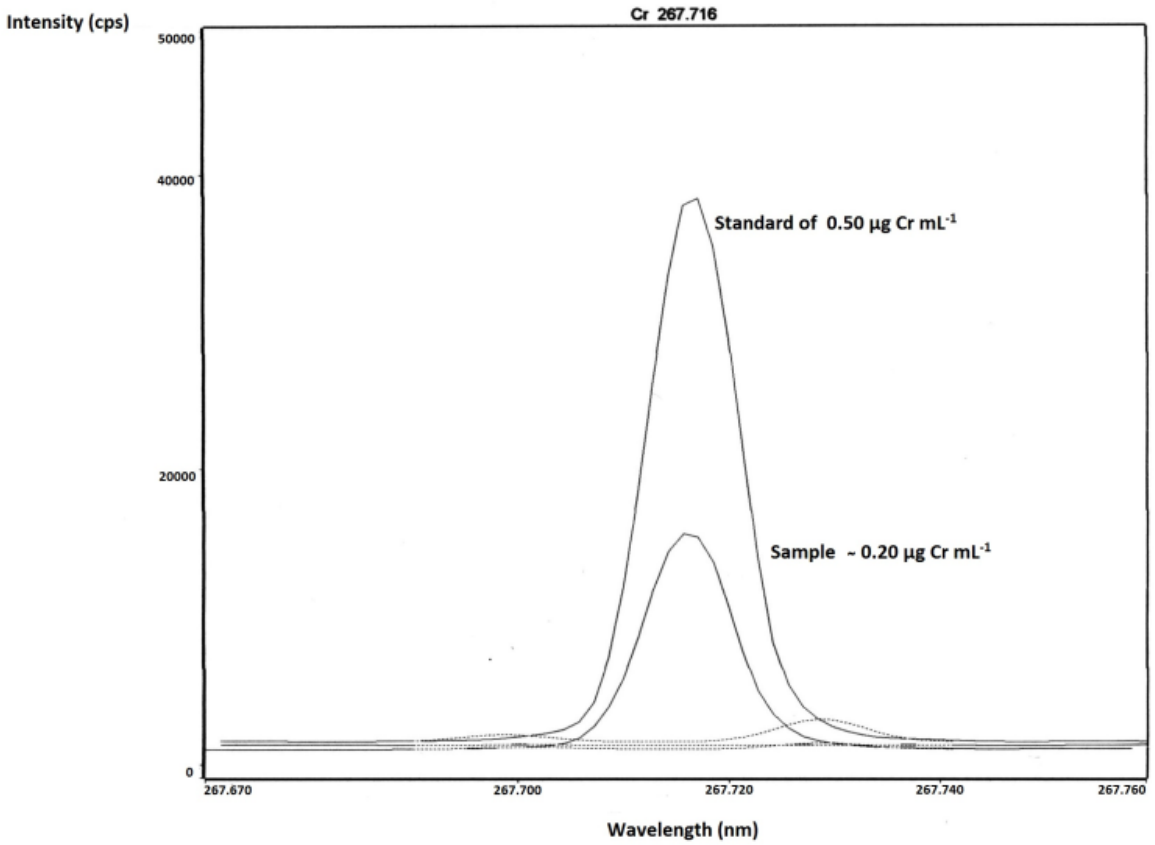


Figure 1. Emission spectra generated by introduction of a sample solution prepared using Procedure (II) and a 0.50 µg Cr mL^{-1} calibration standard. Dotted lines reflect procedural blanks; that with a peak at 267.73 nm arises from a processed sample.

Results for Cr leached from a Cr_2O_3 pigment following application of all six sample preparation procedures are presented in Table 2. Information supplied by the manufacturer and from previous analyses showed 75% w/w purity of Cr_2O_3, equivalent to 513 mg g^{-1} Cr in the pigment. It is evident that these procedures liberated varying amounts of Cr from the pigment, spanning a significant range of 7.5 µg g^{-1} to 2.34 mg g^{-1}. Procedure I, using only water, was the least effective in solubilizing any specie of Cr, as expected, whereas 0.01 mol L^{-1} Na_3PO_4 (procedure IV) interacted with the pigment, decreasing the particle size to the degree that a double filtering through the 0.45 µm membrane was required to remove visible particulates. Although all procedures leached Cr from the pigment to some degree, the presence of Cr(VI) was not detected by Vis spectrophotometry, indicating no Cr(VI) is present in this tested sample of pigment, nor is there any detectable interconversion or oxidation reaction of Cr(III) to Cr(VI) arising from the use of the various leaching procedures.

The varying quantities of Cr leached by the different solutions are indicative of some degree of chemical reaction which either partially “digests” the Cr_2O_3 or, as the sample was subjected to a similar degree of physical agitation in all cases, results in a degradation of particle size below 0.45 µm such that it becomes part of the “solubilized” fraction and is detected with the ICP OES system. Thus, the degree of leaching likely reflects this solubility of Cr_2O_3 in the leaching media. Although insoluble in water, Cr_2O_3 reacts with acid to produce salts of hydrated chromium ions such as $[Cr(H_2O)_6]^{3+}$ and is also attacked by alkali to yield salts of $[Cr(OH)_6]^{3-}$.

Table 2. Cr leached from Cr_2O_3 using various procedures, n=3.

Procedure	ICP OES leached Cr, µg g^{-1}	UV/Vis Cr(VI), µg g^{-1}
I	7.5 ± 0.2	< 1.0
II	47.8 ± 0.2	
III	27.5 ± 0.2	
IV	2340 ± 150	
V	1054 ± 46	
VI	103.5 ± 1.0	

< limit of quantification (µg g^{-1}).

Influence of sample matrix and extraction medium on absorption wavelength

During complexation, Cr(VI) oxidizes diphenylcarbazide (DPC) to 1,5 diphenylcarbazone (DPCO) while being reduced to Cr(III)DPCO, i.e. [20]:

$$2Cr(VI) + 3\ DPC \longrightarrow 2Cr(III) + 3DPCO + 6H^+ \quad (2)$$

$$Cr(III) + DPCO \longrightarrow [CrDPCO]^+ + 2H^+ \quad (3)$$

Classical spectrophotometric measurements described in the literature following the reaction of Cr(VI) with DPC typically utilize absorbance in the region 530 - 540 nm for quantitation [7,8,21]. Figure 2 illustrates the effect of the six different extraction media spiked with 0.5 µg mL^{-1} Cr(VI) on the absorption band. The band head at 540.0 nm was confirmed as being useful for analytical measurements and reveals no impact of the measurement medium on the results.

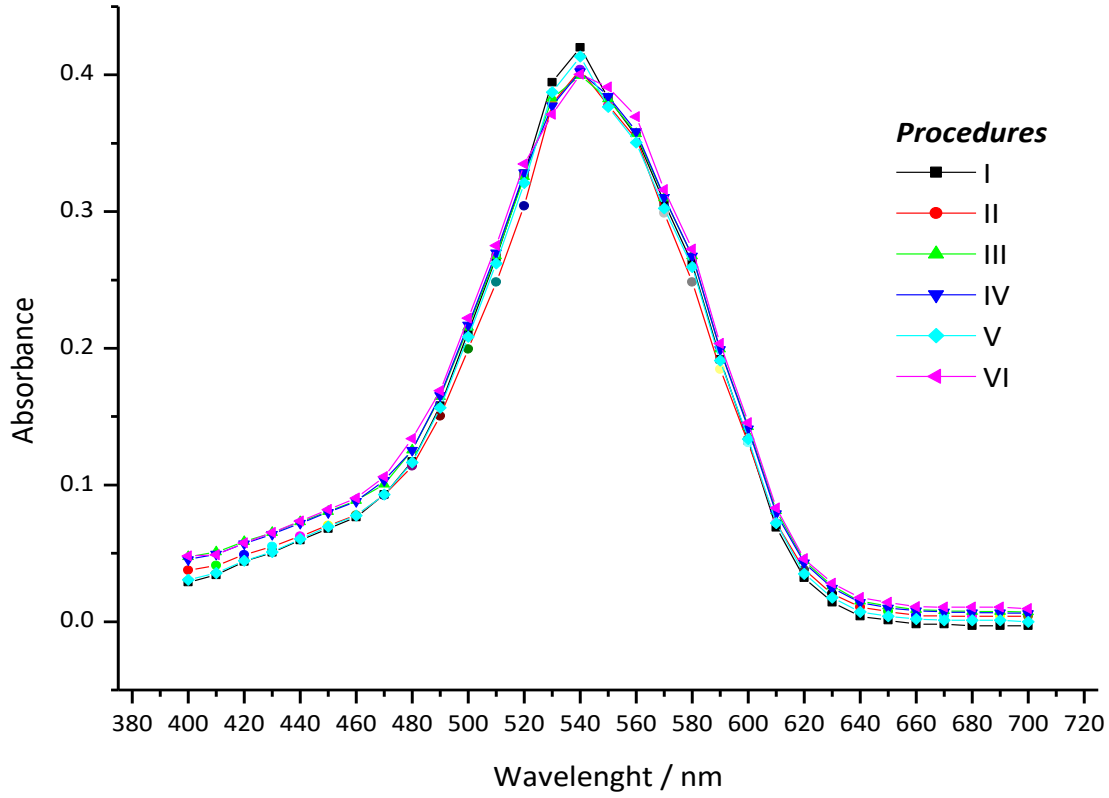


Figure 2. Effect of various Cr_2O_3 pigment extraction media on absorbance band structure. Each solution was spiked with 0.5 µg mL^{-1} Cr(VI).

Evaluation of the development time and stability of the $[CrDPCO]^+$ complex

The time for development and stability of the $[CrDPCO]^+$ complex was examined and is shown in Figure 3. It is evident that the kinetics of formation of the complex varied over the initial 5 minutes in the different extraction media, with the rate of formation of the complex being: V > IV > I ~ VI > III > II. After 5 min the reaction appears complete. In a simple aqueous solution (extraction medium I) the mean absorbance value by the complex remains unchanged or with no significant variation over the course of an hour. The other five extraction media show a small decrease in absorbance, notable after 30 min. A development time of 5 min and stabilization period of 30 min were thus fixed for all subsequent experiments.

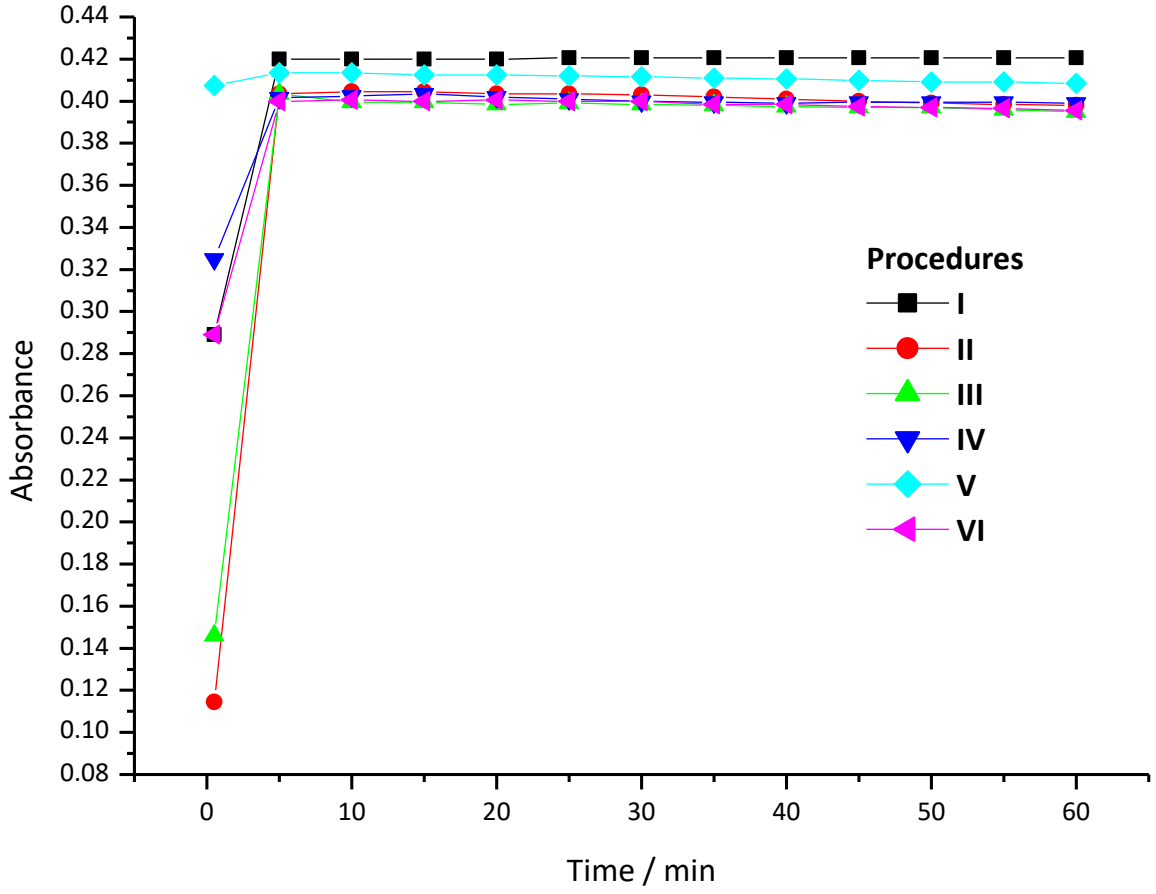


Figure 3. Effect of time on development of the $[CrDPCO]^+$ complex. Different procedural media spiked with 0.5 µg mL^{-1} Cr(VI).

Evaluation of the Influence of Cr(III) on formation of the $[CrDPCO]^+$ complex

The effect of the presence of Cr(III) ions on Cr(VI) in the complexed form $[CrDPCO]^+$ in the six extraction media was examined. Results are shown in Figure 4. Standards of 0.5 µg Cr(VI) mL^{-1} were prepared in the six extraction media and spiked with concentrations of Cr(III) ions ranging from 0.0 to 15.0 µg mL^{-1}.

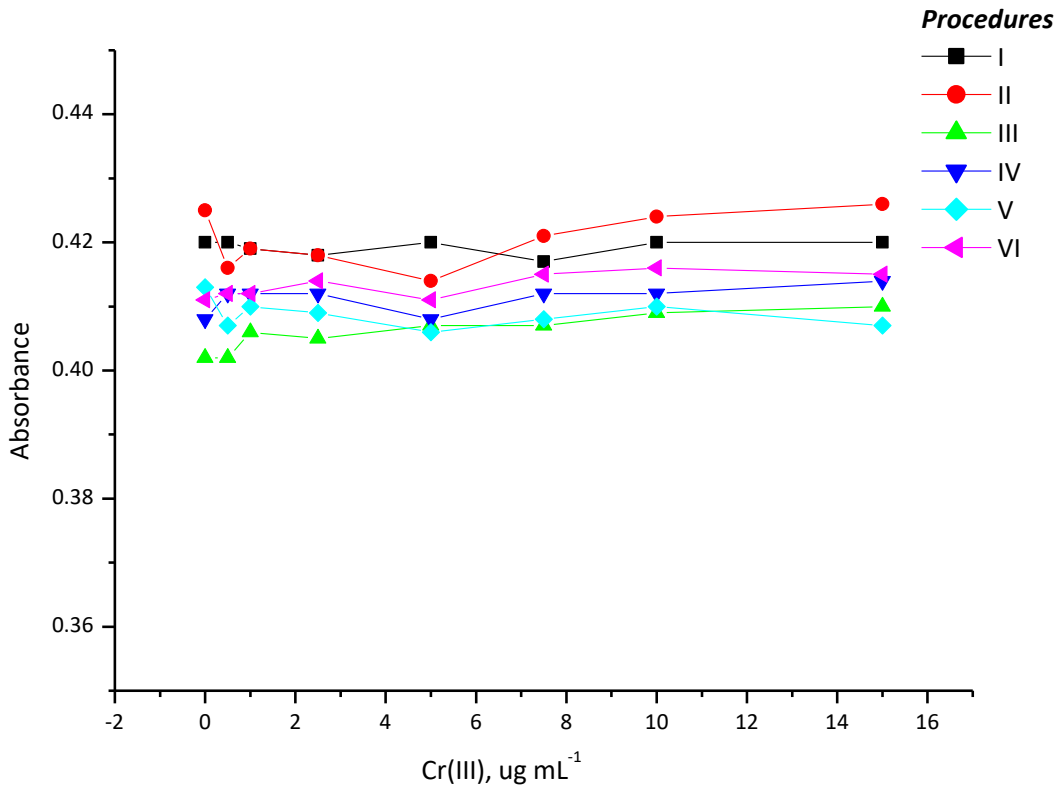


Figure 4. Effect of added Cr(III) in different extraction media. Standard of 0.5 µg mL^{-1} Cr(VI) spiked with varying concentrations of Cr(III) and subjected to extraction procedures I to VI.

No significant change in the absorbance by the Cr(VI) standard solutions occurs despite the presence of excess Cr(III) ion. The determined value of the concentration of the standard showed 99.5 - 100.5% recovery, demonstrating no evidence of impact of Cr (III) ions influencing the formation of the $[CrDPCO]^+$ complex, irrespective of the extraction procedure applied.

Recovery yield for soluble and insoluble Cr(VI) species

Samples of green Cr_2O_3 pigment were enriched with concentrations of 1.0 µg mL^{-1} Cr (VI) in the form of either K_2CrO_4 or $PbCrO_4$ salt (as models of soluble and water insoluble salts, respectively), and subjected to the different procedures under study. Recovery of Cr(VI) was also assessed from the matrix-free pure salts (absence of pigment matrix) submitted to the different procedures. Table 3 summarizes the results obtained.

Table 3. Recovery of 1.0 µg mL^{-1} Cr(VI) species, n=3.

Procedure	Cr(VI), µg mL^{-1}			
	Without Cr_2O_3 pigment		With Cr_2O_3 pigment	
	K_2CrO_4	$PbCrO_4$	K_2CrO_4	$PbCrO_4$
I	1.04 ± 0.02	< 0.01	0.06 ± 0.02	< 0.01
II	1.08 ± 0.04	1.01 ± 0.01	0.60 ± 0.06	0.41 ± 0.10
III	0.96 ± 0.02	1.01 ± 0.06	0.60 ± 0.04	0.37 ± 0.02
IV	1.06 ± 0.02	1.04 ± 0.01	0.68 ± 0.02	0.07
V	1.00 ± 0.02	0.66 ± 0.01	0.16 ± 0.05	0.07
VI	1.02 ± 0.02	0.89 ± 0.02	0.86 ± 0.02	0.80 ± 0.18
Recovery range, %	96-108	0-104	6-86	0-80

< limit of quantification (µg mL^{-1}).

The presence of the Cr_2O_3 pigment matrix clearly influences the determination of soluble and insoluble Cr(VI) species for all evaluated procedures. Considering only the soluble and water insoluble salt forms, in the absence of the matrix, recoveries showed acceptable values with the exception of use of only deionized water (Procedure I) for the insoluble form of Cr(VI), as expected. In the presence of the Cr_2O_3 pigment matrix, Procedure I provided a recovery of only 6% for the soluble form, whereas for the insoluble form this remained unchanged and was below the limit of quantification [18]. Similar characteristics were observed for Procedure V which uses an acidic extraction medium (IRSA16 method) [12,13], with recovery of the soluble species being 16% and of the insoluble species 7%, confirming results obtained by Bruzzoniti and coauthors [11] in their study of cosmetic pigment samples. Procedures II and III, based on the EPA 3060A methodology [9] without and with Mg^{2+} ions respectively, provided similar results with recovery of 60% for the soluble form and approximately 40% for the insoluble form of Cr(VI). The presence of Mg^{2+} ions serves to avoid the process of reduction/oxidation of Cr(VI) in the test sample [9]. In this case it is evident that there is no need for the addition of Mg^{2+} to this type of matrix, since the results obtained were similar using either method. However, for quantitative purposes, it is clearly not possible to use these procedures for analysis of insoluble species in this Cr_2O_3 pigment matrix due to the low recovery of the analyte [9,10]. Procedure IV, based on extraction with Na_3PO_4, provided 68% recovery of the soluble form, but very low recovery (7%) of the insoluble form. These results are similar to those reported by Bruzzoniti and coauthors [11]. Several attempts were made to improve the spike recovery of both soluble and insoluble forms of chromium using Procedure VI, including increased vortex stirring time, color development time and increasing the concentration of Na_2CO_3 extraction reagent. However, no improvement was achieved, possibly indicative of a small interconversion of the species. However, only with minor variations (vortex agitation and development time), this methodology proposed by Panichev and colleagues [14,15], proves to be more efficient than all others tested. Additionally, in accordance with U.S. Food and Drug Administration guidelines [22], recoveries of 86% and 80%, for the extraction of soluble and insoluble species in the presence of the Cr_2O_3 pigment matrix are considered acceptable. This procedure was therefore selected as the most suitable for the determination of Cr(VI) species present in the Cr_2O_3 pigment.

Figures of merit: calibration with different procedures

Correlation coefficients > 0.999 characterize the linear portions of the calibration functions spanning 0.05 – 1.50 μg mL^{-1} Cr(VI) for all sample preparation procedures, demonstrating adequate linearity. A significant matrix effect was not evident, with slopes of the calibration function differing by less than 5% (0.7917 to 0.7550) as a slight suppression in the matrix matched medium compared to standards prepared in high-purity water. Nevertheless, standards were prepared in matrix matched reagent media to obviate this slight bias. Accounting for sample preparation (250 mg/25 mL), the limit of detection (LOD), defined as 3 times the standard deviation of 10 replicate measurements of the blank divided by the slope of the calibration function, and the limit of quantification (LOQ), defined as 10 times the standard deviation of 10 replicate measurements of the blank divided by the slope of the calibration function, are 0.3 μg g^{-1} and 1.0 μg g^{-1} respectively [23], satisfying required legislated limits of 1 μg g^{-1}[5,10].

Application to real samples

Procedure VI was applied to the analysis of four commercial samples of Cr_2O_3 pigment provided by a local cosmetic company. Only one showed detectable Cr(VI) whereas the other three presented levels below the required limit of 1.0 μg g^{-1} [5,10]. This sample was selected for further in-house study. Approximately 100 g was sieved through a 35 mesh stainless steel sieve to improve homogeneity. After sieving, this sample was stored in glass bottles in a desiccator at less than 30ºC. No further sub-division was performed, as the sample was relatively small [24]. The sample was then analyzed under conditions of repeatability and reproducibility (identical experimental conditions but at a different time, i.e., one month) using Procedure VI. Both precision and recovery of the method were evaluated. Since certified reference materials for this type of material do not exist, the objective of this evaluation was to determine whether this sample could be utilized in the laboratory as a quality control material [24]. Results are presented in Table 4 and demonstrate fit for purpose accuracy with relative standard deviations of 2.4% for repeatability and 1.9% for reproducibility conditions, in accordance with U.S. Food and Drug Administration guidelines [22]. Similarly, acceptable recoveries of 50 μg g^{-1} spikes of soluble Cr(VI) added to the sample were 88% under repeatability and 84% under reproducibility conditions [22]. Recoveries at the 100 μg g^{-1} spike level of insoluble Cr(VI) were 83% under repeatability and 83% under reproducibility conditions and are biased slightly below the recommended 90 %

level advocated by the USFDA [22], the methodology may find use for investigation of insoluble species. These results demonstrate the accuracy of Procedure VI methodology when the target is soluble species. The final average concentration of 16.6 ± 0.6 µg g^{-1} (n=14) Cr(VI) reveals that this particular test sample is of a batch that cannot be used as a pigment for cosmetic fabrication. However, it may find use in the testing laboratory as an internal quality control material and will continue to be examined over the course of at least 2 years to determine its stability in this laboratory.

Table 4. Method precision achieved with commercial samples of Cr_2O_3 pigment.

	Cr(VI), µg g^{-1}					
	Repeatability conditions			**Reproducibility conditions**		
n	Sample	Sample spike[1] K_2CrO_4	Sample spike[2] $PbCrO_4$	Sample	Sample spike[1] K_2CrO_4	Sample spike[2] $PbCrO_4$
1	17.9	42.9	78.8	16.2	41.3	90.7
2	17.2	43.7	81.4	16.0	42.1	78.5
3	16.6	43.7	85.0	16.7	41.0	84.2
4	16.3	44.4	78.0	16.1	42.8	80.5
5	16.4	43.1	90.7	16.0	42.0	77.6
6	16.9	44.7	79.1	16.1	41.5	83.4
7	17.2	45.7	88.4	16.5	41.9	87.3
Average	16.8 ± 0.4	44.0 ± 1.0	83.1 ± 5.0	16.2 ± 0.3	41.8 ± 0.6	83.2± 4.7
RSD, %	2.4	2.2	6.1	1.9	1.4	5.7
%, Rec.	----	88	83	---	84	83

[1] 50 µg g^{-1} spike
[2] 100 µg g^{-1} spike

CONCLUSION

Procedure VI, employing a Na_2CO_3 based extraction, proved to be the most efficient, yielding good precision and accuracy when applied to a real sample, as demonstrated by acceptable recoveries of soluble and insoluble Cr(VI) species. The proposed spectrophotometric method is simple, inexpensive, readily available, and conveniently provides for speciation of Cr(VI) in chromium oxide green (Cr_2O_3) pigments used as colorants for cosmetic products that satisfy the legislated limits. Such speciation is not possible using direct determinations by techniques such as ICP OES or F AAS. It has been adopted for routine application by the Paraná Institute of Technology – Tecpar (Curitiba, Brazil) laboratory for this purpose.

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