Matrix isolation with an ion transfer device for interference-free simultaneous spectrophotometric determinations of hexavalent and trivalent chromium in a flow-based system

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Abstract

Chromium speciation by spectrophotometric determination of hexavalent chromium (Cr(VI)) with diphenylcarbazide (DPC) has several problems. These include: (1) the inability to directly detect trivalent chromium (Cr(III)) with DPC, (2) positive interference in Cr(VI) determination by other metal cations and (3) negative interference by any reducing agent present in the sample. These are addressed with an ion transfer device (ITD) in a flow injection analysis system. We previously developed the ITD for electrodialytic separations. Here we separate oppositely charged Cr(III) and Cr(VI) species by the ITD into two different acceptor solutions within ~5 s. The acceptor solutions consist of buffered H₂O₂ to oxidize the Cr(III) to Cr(VI). Then DPC is added to either acceptor to measure Cr(III) and Cr(VI) spectrophotometrically. The system was optimized to provide the same response for Cr(VI) and Cr(III) with limits of detection (LODs, S/N =3) of 0.5 µg L⁻¹ for each and a throughput rate of 30 samples h⁻¹. The ITD separation was also effective for matrix isolation and reduction of interferences. Potential cationic interferences were not transferred into the anionic Cr(VI) acceptor stream. Much of the organic compounds in soil extracts were also eliminated as evidenced from standard addition and recovery studies.

Key words: Electrodialysis, chromium, diphenylcarbazide, spectrophotometry, soil extracts, flow injection analysis

1. Introduction

Spectrophotometric chromium (Cr) determination with 1,5 – diphenylcarbazide (DPC) is widely used. Environmental Cr exists either as cationic trivalent Cr(III), an essential nutrient, or anionic hexavalent Cr(VI), a class I carcinogen as defined by International Agency for Research on Cancer (IARC). The DPC method, now more than a century old [1], can measure toxic Cr(VI) selectively, sensitively, and rapidly. It is approved as a standard method in many countries, e.g., in Japan and the United States. The standard procedure involves batch analysis, the filtered sample solution is mixed with strongly acidic DPC and the absorbance is then measured (typically at 540 nm). The exact reaction pathway of DPC with Cr(VI) has never been unequivocally established but it is believed that the DPC is oxidized by Cr(VI) to diphenylcarbazone, which then reacts with the concomitantly produced Cr(III) to form the observed colored product [2]. In any case, the reaction is rapid at elevated temperature and the colored product formed is stable.

The DPC chemistry has been adapted to the flow analysis format [3]. Sensitivity improvements have been achieved by a long path flow cell [4-6], solid phase spectrometry [7] and thermal lens spectrometry [8]. Oxidation with Ce(IV) [9-11], H₂O₂ [12] or deep UV light [13,14] have been used for conversion of Cr(III) to Cr(VI). Determination after such oxidation gives total Cr ([Cr_T] = [Cr(III)] + [Cr(VI)]) from which Cr(VI), determined without any oxidation, was used to calculate Cr(III) by subtraction. Such subtraction procedures are susceptible to large errors when the Cr(VI) concentration is much larger than that of Cr(III). In the same vein, DPC-based measurement of [Cr(VI)], along with atomic spectrometric measurement of Cr_T has also been used [15].

Because Cr(III) and Cr(VI) are oppositely charged, solid phase extraction (SPE) with ion exchangers that selectively sorb Cr(III) or Cr(VI) can be exploited to selectively measure either or both; DPC chemistry can still be used if the Cr(III) is oxidized to Cr(VI) after elution [16]. Similar strategies are possible with atomic spectrometric techniques as the measurement method; the general area of Cr speciation with SPE has been reviewed [17,18]. Some exotic sorbents for highly selective Cr species collection have also been reported [19,20]. The latter are not commercially available, however, the essential problem with ion exchange based selective sorption is that either Cr(III) or Cr(VI) occurs in real samples in traces with far larger amounts of other ions; ion exchange capacity is often insufficient, especially for saline samples. In addition, prior sample pretreatment including pH adjustment, are typically necessary for applying SPE-based methods.

We have previously reported on electrodialytic matrix isolation/preconcentration for ionic solutes using an Ion Transfer Device (ITD) [21]. While cationic and anionic species are quantitatively transferred to oppositely placed individual acceptor streams by the applied electric field, non-ionic compounds, low mobility species like proteins, and particulate matter are not transferred and are wasted together with the depleted sample. Electrodialytic matrix isolation was demonstrated to be of utility in the determination of anionic species like carboxylic acids [22] and cationic species like heavy metal ions [23] in real samples. Furthermore, the electrodialytic matrix isolation was successfully applied for Cr speciation analysis [24] with sub- $\mu\text{g L}^{-1}$ limits of detection by inductively coupled plasma- mass spectrometry (ICP-MS).

While it is hard to do any better in terms of LODs and the sample throughput rate than the ICP-MS adaptation of the ITD, an ICP-MS is very expensive to own, operate and maintain and is not accessible to all. In the present study, we fully optimize the use of ITD based Cr speciation with DPC-based measurement, with special attention to

matrix isolation and interference in Cr(VI) measurements by metal cations [25-28] and anions [29,30]. Soil extracts were successfully analyzed for Cr(VI) by this approach.

2. Experimental

2.1 Reagents

All reagents were obtained from www.nacalai.co.jp or www.wako-chem.co.jp. Ultrapure water (Milli QTM www.emdmillipore.com) was used in all experiments. Standard solutions of Cr(III) and Cr(VI) were prepared in 1 mM HNO₃ with CrCl₃·6H₂O and K₂CrO₄·6H₂O, respectively. Trace Element Analysis grade HNO₃ was used throughout the experiments. The DPC reagent was prepared by dissolving DPC (0.3 g) in acetone (10 mL) and diluting to 500 mL with 0.2 M H₂SO₄. The carrier/oxidizer, 7.5 mM H₂O₂ in pH 8.5 borate buffer, was prepared by adding H₂O₂ (0.43 mL, 30% w/v) into borate buffer (500 mL, pH 8.5; 1.35 g of H₃BO₃ in 500 mL water, pH adjusted with KOH). Metal ion standards for the interference study were prepared with NiCl₂·6H₂O, CoCl₂·6H₂O, ZnCl₂, MnCl₂·4H₂O, Pb(NO₃)₂, FeCl₃·6H₂O, CuCl₂·2H₂O, NH₄VO₃, and (NH₄)₆Mo₇O₂₄·4H₂O.

2.2 Ion transfer device

Details of the ITD structure were previously reported [23, 26]. The schematic diagram is in Fig. 1a. Five solution layers were stacked vertically, separated by appropriate membranes that allow passage of ions. The sample is introduced into the center channel while acceptor channels, separated respectively by cation and anion exchange functionality modified regenerated cellulose membrane (CFDM and AFDM, see [24, 26]) flank the sample channel on the top and bottom. The outermost channels, called isolator channels serve to prevent direct electrode contact (and hence any redox conversion) of the acceptor channel contents. The outermost channels are furnished with Pt screen electrodes (0.07 mm wire diameter, 100 mesh, www.nippon-clever.co.jp). The cation/anion acceptor and cathode/anode isolator channels were separated by anion and cation exchange membranes (AEM and CEM, Selemion® DSV and CMV, www.agc.com), respectively. The channels themselves were prepared by pressing Parafilm® on nylon screen (150 mesh, thickness 106 µm, www.nippon-clever.co.jp). The dimensions of the flow-through portion of each channel were 5 mm width × 40 mm length × 0.13 mm height. Isolator channels were machined with 0.2 mm depth on the top and bottom PVC plates.

Peristaltic pumps were used for pumping sample, carrier, and isolator solutions while syringe pumps were used for acceptor solutions. Accurate flow rates are essential for sample and acceptor solutions. However, chromium species, especially Cr(III), is easily adsorbed on the glass syringe surface. The sample solution was therefore pumped peristaltically with Pharmed[®] pump tubes. Sample/carrier and acceptor solutions (all 10 mM HNO₃) were pumped at 0.20 mL min⁻¹ and isolator solutions (30 mM HNO₃) were pumped at 3 mL min⁻¹. A constant 15 V DC was applied between the electrodes. The flow rates, concentrations, and applied voltage has been optimized as previously described [26]. The anode and cathode acceptor solutions were individually introduced into the flow injection analysis system.

2.3 DPC based flow analysis system

Chromium in either ITD acceptor effluent was determined with the flow injection analysis system based on DPC chemistry (Fig. 1b). Automated sample injectors (Cheminert C22Z-3186D, www.vici.com, included 50 μL loop) each for Cr(III) and Cr(VI) were serially placed in the carrier stream of buffered H_2O_2 . The stream next flowed through a heated reaction coil (60 $^\circ\text{C}$, 400 μL , nominal t_R 30 s) to complete the oxidation by hydrogen peroxide and was then merged with the acidic DPC reagent flow and followed by another reaction coil (60 $^\circ\text{C}$, 350 μL , nominal t_R 13 s) and then through an absorbance detector (Spectroflow 757, Kratos) set at 540 nm. All system tubing, including the heated reactors, were composed of 0.5 mm i.d. 1.6 mm o.d. PEEK tubing (www.glsience.com); PEEK has approximately the same thermal conductivity (0.25 W/m K) as the more commonly used PTFE but shows less adsorption of the DPC reaction product). The two heated coils were part of an integrated arrangement that consisted of the two tubular reactors, a heating element and a thermocouple (621–2158, <http://jp.rs-online.com/>) all potted in a block of low-melting bismuth-tin alloy (Wood's metal) [31].

For soil extract measurement, the Japanese standard method measures only Cr(VI). The extraction conditions uses pH 5.8–6.3. To detect Cr(VI) only, the FIA system was simplified by omitting the Cr(III) injector and the oxidation reactor (components in the dotted enclosure) in Fig. 1b. The carrier and the anode acceptor solutions were both 10 mM HNO_3 .

2.4 Preparation of soil extract

Soil samples used in the present work came from near the Shirakawa river (Kumamoto, Japan) in winter from three locations: (a) under a tree, (b) grassy lawn, and (c) playground. The samples were treated following prescribed Japanese environment agency protocols [32]: The collected samples were equilibrated overnight in a constant humidity environment (20% RH, SD-3, www.as-1.co.jp). After removing large particles by passing through a 2-mm sieve, 500 mL water was added to 50 g sample and the pH of the stirred suspension was adjusted to 6 with dilute HCl. The mixture was placed in a 1-L plastic bottle and shaken on a mechanical shaker (200 oscillations per minute) for 6 h. The mixture was allowed to settle for 20 min and an aliquot of the supernatant then centrifuged for 20 min at 3,000 rpm (SPN-400, www.shimadzu.com). The clear supernatant was filtered through a 0.45 μm PTFE filter and the filtrate analyzed by the present system. The spectra of the filtrate in quartz cuvettes were also measured with a double beam UV-VIS spectrophotometer (UV-3600, www.shimadzu.com).

3 Results and Discussion

3.1 Optimization for simultaneous FIA analysis of Cr(III) and Cr(VI)

In the present study, both forms of Cr were measured with DPC chemistry. As Cr(III) does not react with DPC, an oxidation procedure is required. We considered the three different oxidants that have mainly been used for this purpose: Ce(IV) [11], deep-UV radiation [13, 14], and H_2O_2 [12, 16]. Oxidation with Ce(IV) requires acidic conditions, under these conditions, Cr(VI) formed also functions as an oxidant and can be lost by reaction with slowly oxidized material such as organic acids [11] that may be present in a soil extract. Oxidation with UV-radiation was particularly attractive because no reagent is involved. However, when a Cr(III) standard (1 mg

L^{-1} Cr(III) in 0.1 M HNO_3) flowing through a planar channel (8 mm width $\times$ 50 mm length $\times$ 0.125 mm depth) at 0.2 mL/min (nominal t_R 15 s) was irradiated via a quartz window with a Hg pen lamp (manufacture specified radiant flux $30 \mu\text{W}/\text{cm}^2$ @ 30 cm from source, P/N 81-1025-01, www.bhkinc.com) placed immediately atop, the conversion efficiency was only 54 %. The UV oxidation efficiency depends on the solution pH; quantitative conversion has been reported at pH 3.2 [14]. However, any need to adjust pH decreases sample throughput and formation of Cr(VI) under acidic condition will make it susceptible to reduction by any oxidizables present. Also, considering that real samples will have other and varying amounts of UV absorbing substances, this did not seem to be a practical approach without performing standard addition. On the other hand, H_2O_2 could convert from Cr(III) to Cr(VI) quantitatively under mildly alkaline conditions in which Cr(VI) is not a strong oxidant and is not easily lost. The H_2O_2 concentration optimization result appear in Fig. 2a. The standard solutions ($50 \mu\text{g L}^{-1}$ each) were directly introduced into the sample loops. With 10 mM HNO_3 as the carrier, essentially only Cr(VI) responded (left panel, Fig. 2a). But with H_2O_2 in a pH 8.5 borate buffer the conversion of Cr(III) to Cr(VI) started and the highest value was obtained with 6.0 mM H_2O_2 . Note that at higher H_2O_2 concentrations the response decreases and even at the optimum 6.0 mM H_2O_2 the response of Cr(VI) is $\sim 15\%$ less than that with a 10 mM HNO_3 carrier. This is because after acidification with the DPC reagent some Cr(VI) can be lost by H_2O_2 acting as a reductant and this loss is dependent on the residual H_2O_2 concentration. While others have suggested a carbonate buffer [16] as the medium for the H_2O_2 oxidation of Cr(III), this is incompatible with the present system because of formation of CO_2 bubbles after addition of the acidic DPC solution.

The reaction temperature was also optimized. In the present study, reactors for oxidation and color development were integrated into one heated block. Around room temperature (20°C), the Cr(III) response was less compared to that of Cr(VI). However, the two became equal at 40°C (see the green trace in Figure 2b for the response ratio) and remained equivalent until 80°C , the highest temperature studied. However, the absolute response increased until 60°C and did not increase further thereafter. A reactor temperature of 60°C was henceforth chosen.

3.2 Response characteristics

The acceptor effluents from the ITD were injected into the optimized FIA system. The system output for $10 \mu\text{g L}^{-1}$ Cr(III), $10 \mu\text{g L}^{-1}$ Cr(VI), and a mixture containing $10 \mu\text{g L}^{-1}$ of each introduced into the ITD is shown in Fig. 3. The ITD could quantitatively transfer the Cr(III) and Cr(VI) into the different acceptor solutions. The first peak is absent if there was no Cr(VI) and similarly, the second peak was absent if there was only Cr(VI). The responses for Cr(III) and Cr(VI) were statistically equivalent: peak heights for $10 \mu\text{g L}^{-1}$ of Cr(III) and Cr(VI) were 3.93 ± 0.04 and 3.97 ± 0.03 mAU, respectively ($n = 3$). Calibration behavior was studied with the mixed standards up to concentrations of $50 \mu\text{g L}^{-1}$ each; a response FIAGram is also shown in Fig. 3. The calibration equations were:

$$\text{Cr(III) Peak height, mAU} = (0.405 \pm 0.008) \times [\text{Cr(III), } \mu\text{g L}^{-1}] - (0.18 \pm 0.23) \quad \dots(1)$$

$$\text{Cr(VI) Peak height, mAU} = (0.406 \pm 0.007) \times [\text{Cr(VI), } \mu\text{g L}^{-1}] - (0.14 \pm 0.22) \quad \dots(2)$$

Limits of detection based on the $S/N=3$ criterion were both $0.5 \mu\text{g L}^{-1}$ for Cr(III) and Cr(VI). Independent measurement of Cr species as carried out here will produce more accurate results compared to subtraction-based approaches.

3.3 Interferences

Interference by many cationic heavy metals in DPC-based determination of Cr(VI) is well documented [4, 25-30]. Interference studies were performed with the several kinds of metal cations and oxometal anions for the present system. Figure 4 shows that cationic metals Fe^{3+} , Cu^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} and Pb^{2+} at the 10 mg L^{-1} level had no discernible effects on the response of $10 \mu\text{g L}^{-1}$ Cr(VI). This is understandable as the cations are not collected in the anode acceptor where the Cr(VI) ends up. Without the ITD, Cu^{2+} interferes at concentrations as low as 1 mg L^{-1} and Fe^{3+} interferes by 5 mg L^{-1} . Importantly vanadate and molybdate are oxometallates just like Cr(VI) and end up together with Cr(VI). Tolerance to these ions is limited; while there is no effect at $100 \mu\text{g L}^{-1}$ V or Mo, there is a discernible interference at concentrations $\geq 1 \text{ mg L}^{-1}$. The concentration of these ions in drinking water and soil extracts has been reported to be in the range of $0.003\sim 0.54$ and $0.06\sim 22.7 \text{ mg L}^{-1}$ for V and Mo, respectively [33,34]. Realistically, the interference from vanadate is not therefore likely to be significant. We also found in batch experiments that the DPC-vanadate product is unstable with time. For example, 1 mg L^{-1} V as vanadate caused a +12.5% interference in the measurement of $250 \mu\text{g L}^{-1}$ Cr. After 15 min, this decreased to a +5% interference. If high V is an issue, a stopped-flow approach where the peak apex is arrested in the cell and a decrease in absorbance with time is monitored (and projected to completion) will be worthy of investigation. For Mo, others have reported [35] that the addition of oxalic acid to the DPC reagent formulation as a masking agent eliminates the interference: we have confirmed that this is applicable in the presently proposed system.

For Cr(III) measurement, Fe^{3+} and Cu^{2+} interfered above 5 and 1 mg L^{-1} , respectively, none of the other metal species studied interfered.

3.4 Matrix isolation for soil extract analysis

An important advantage of the ITD for sample pretreatment is matrix isolation. This is more important to spectrometric measurements than element-specific ICP-MS. The evaluation of the matrix isolation efficiency was studied for the determination of Cr(VI) in soil extracts.

Chromium in soil leachates need to be analyzed for environmental impact evaluations. Soil extraction methods have been standardized by official agencies in different countries and may differ between countries. The Japanese standard method [32] prescribes extraction with purified water after pH adjustment to 6 with HCl. The method is for Cr(VI) determination. At this pH, Cr(VI) does not function as oxidant. On the other hand, at this pH Cr(III) is not extractable. In preliminary experiments, we have tested spike recoveries of both Cr(VI) and Cr(III) added to soil with the present analysis system. The recoveries of Cr(III) was essentially zero. Based on the thermodynamic as well as experimental data, approximately $50 \mu\text{g Cr L}^{-1}$ can be dissolved at pH 6 in water [36]. However, at this pH, the carboxylic acid groups in soil humus will be negatively charged and Cr(III) will be tightly

bound - Cr(III) is always far less bioavailable and mobile in soils compared to Cr(VI) [37]. While Cr(III) does form fulvic acid complexes (and presumably humic acid complexes), they are retained by ultrafilters [38] and as such will not be considered soluble and are very unlikely to pass through the present dialysis membranes in a short period. Moreover, such complexes will be essentially neutral. We tested low molecular weight organic ligands e.g., ascorbate, glutamate, mandelate, citrate, cysteine, serine, pyruvate and oxalate, considered as possible organic ligands in soil extracts [39]. The fully formed complexes are assumed to be negatively charged. However, when we separately introduced individual Cr(III):ligand mixtures (1:5 molar ratio, containing 1 mg Cr(III) L⁻¹, pH adjusted to 6) into the ITD, no Cr was found in acceptor (+) channel but some Cr(III), much less than quantitative, was observed in acceptor (-) channel, presumably due to the dissociation of the complex. When Cr(III)-organic complex solutions were directly introduced into FIA system without ITD separation, the response also depended on the particular ligand used, suggesting considerable kinetic differences in the oxidation of various Cr(III)-organic complexes by H₂O₂ [40]. Clearly, the quantitative determination of Cr(III) in its organic complexes as well as their behavior in ITD based separation - determination systems need more study. Presently, we focused on the evaluation of matrix isolation efficiencies for the determination of Cr(VI) in soil extract.

Soil extract contain many colored organic compounds such as humic acids and often also very fine particulate matter that passes through a 0.45 µm filter. Both interfere with the accuracy of low level absorbance measurements. The Japanese industrial standard (JIS) method utilizes a blank measurement in which a small amount of methanol is added to the acidified sample (which then reduces Cr(VI) to Cr(III) that does not react with DPC). In the flow analysis literature, blank subtraction where the blank is obtained without DPC addition has been suggested [41]. Another article suggests generation of a blank in-line by adding ascorbic acid to reduce Cr(VI) to Cr(III) [42]. All of these approaches involve additional steps. The importance of this blank preparation can be observed in Fig 5a. All three of our filtered soil extract samples had significant absorbance at 540 nm. Sample B that displayed the highest background absorbance would have registered 150 µg L⁻¹ Cr(VI) if this blank was not corrected for. In contrast, in the present study, the ITD essentially eliminated the interference without having to take separate steps to prepare an appropriate blank and subtract (with the attendant problems of subtraction methods). The results of standard addition to the soil extract samples without using the ITD are shown in Fig. 5b.

The relevant equations for the respective samples without ITD treatment were:

$$\text{Standard: Abs., mAU} = (0.123 \pm 0.003) [\text{Cr}] + (0.111 \pm 0.003)$$

$$\text{Sample A: Abs., mAU} = (0.125 \pm 0.002) [\text{Cr}] + (0.769 \pm 0.054)$$

$$\text{Sample B: Abs., mAU} = (0.050 \pm 0.008) [\text{Cr}] + (12.5 \pm 0.231)$$

$$\text{Sample C: Abs., mAU} = (0.102 \pm 0.004) [\text{Cr}] + (5.93 \pm 0.128)$$

The equations for the same samples with ITD treatment were:

$$\text{Standard : Abs., mAU} = (0.114 \pm 0.003) [\text{Cr}] + (0.405 \pm 0.102)$$

$$\text{Sample A: Abs., mAU} = (0.117 \pm 0.003) [\text{Cr}] + (0.314 \pm 0.076)$$

$$\text{Sample B: Abs., mAU} = (0.114 \pm 0.004) [\text{Cr}] + (0.802 \pm 0.109)$$

$$\text{Sample C: Abs., mAU} = (0.117 \pm 0.003) [\text{Cr}] + (0.632 \pm 0.080)$$

It is not just that large intercepts, as may be expected from the direct spectral absorption measurements, are observed, the greater the amount of the absorbing impurities, the lower is the slope of the best-fit line, suggesting that these absorbing impurities are oxidizable and consume Cr(VI) and the prescribed blank subtraction methods will not really be effective. The same experiment done with the ITD in contrast led to identical standard addition slopes for all samples and essentially reduced the intercept indistinguishable from that of the standard.

4. Conclusion

We have successfully developed a DPC-colorimetry based flow analysis system capable of speciating Cr(VI) and Cr(III) using an ITD. No blank subtraction methods are needed in real soil extract samples containing absorbing impurities. Matrix isolation by the ITD could be generally useful for elimination of interference in other ionic solute determinations. The present system can be used in the field. We are presently working on further enhancing the sensitivity and expect this to become an affordable attractive tool for monitoring environmental Cr.

Acknowledgments

This study was supported partially by Japanese Environment Research and Technology Development Fund (5RF-1602), the Japan Science and Technology, Development of Systems and Technology for Advanced Measurement and Analysis program (SENTAN), and partially by the Steel Foundation for Environmental Protection Technology. Participation of PKD was made possible by a grant from the US National Science Foundation (CHE-1506572).

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Fig. 1 Schematic diagram for the flow system. PP: peristaltic pump, SP: syringe pump, E: electrode, AEM: anion exchange membrane, CFDM: cation exchange functional group introduced dialysis membrane, AFDM: anion exchange functional group introduced dialysis membrane, FIA: flow injection analysis, D: Detector

Fig. 2. Effect of H_2O_2 concentration (a) and reactor temperature (b). The left panel of (a) shows 10 mM HNO_3 as carrier without addition of H_2O_2 . The temperature and H_2O_2 concentration for (a) and (b) were 60 $^{\circ}\text{C}$ and 7.5 mM, respectively. The concentration of test solutions was 50 $\mu\text{g L}^{-1}$.

Fig. 3 Response charts and calibration curves obtained with ITD separation and DPC based FIA system

Fig 4. Interference from co-existing ions on the determination of $10 \mu\text{g L}^{-1}$ of Cr(III) and Cr(VI). The direct was obtained without ITD separation and $10 \mu\text{g L}^{-1}$ of Cr(VI) was determined. Acceptor (+) and (-) were obtained with ITD separation and stand for cathode and anode sides, respectively.

Fig. 5. Spectral absorption of samples and DPC reaction products and standard addition results for soils extracts. (a) Spectra of soil extracts and DPC-Cr products, and (b) standard addition results without ITD separation and (c) with ITD separation.