



Correlation between bulk- and surface chemistry of Cr-tanned leather and the release of Cr(III) and Cr(VI)



Yolanda S. Hedberg^{a,b,*}, Carola Lidén^b, Inger Odnevall Wallinder^a

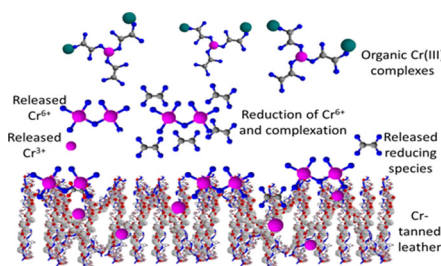
^a KTH Royal Institute of Technology, School of Chemical Science and Engineering, Department of Chemistry, Division of Surface and Corrosion Science, SE-10044 Stockholm, Sweden

^b Institute of Environmental Medicine, Karolinska Institutet, Box 210, SE-17177 Stockholm, Sweden

HIGHLIGHTS

- Released reducing/complexing leather-specific species can reduce released Cr(VI).
- No co-released species enable the formation of Cr(VI) in solution.
- The major Cr species released from leather in phosphate buffer was Cr(III) (>82%).
- No Cr(VI) was released into artificial sweat.

GRAPHICAL ABSTRACT



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ABSTRACT

About 1–3% of the adult general population in Europe is allergic to chromium (Cr). The assessment of the potential release of Cr(III) and Cr(VI) from leather is hence important from a human health and environmental risk perspective. The Cr(VI) content in leather was recently restricted in the European Union. The aim of this study was to assess possible correlations between the bulk and surface chemistry of leather, released Cr(III) and Cr(VI), and capacities of co-released leather specific species to reduce and complex released Cr. Four differently tanned leathers were characterized by scanning electron microscopy with energy dispersive spectroscopy, X-ray photoelectron spectroscopy, attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, and the diphenylcarbazide colorimetric method. Their characteristics were compared with results on Cr(III) and Cr(VI) release into artificial sweat (ASW, pH < 6.5) and phosphate buffer (PB, pH 7.5–8.0), measured by means of spectrophotometry and atomic absorption spectroscopy. Co-released leather-specific species were shown to reduce Cr(VI), both in ASW and in PB. Their reduction capacities correlated with findings of the surface content of Cr and of released Cr. Leather samples without this capacity, and with less aromatic surface groups visible by ATR-FTIR, revealed Cr(VI) both at the surface and in solution (PB).

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1. Introduction

Contact allergy to chromium (Cr) is the third most common metal allergy, preceded by nickel (Ni) and cobalt (Co), and affects approximately 1–3% of the adult general population [1]. Leather has since the 1990s attracted increasing attention as a cause of Cr allergy and dermatitis. This trend is particularly pronounced for women, indicated by an increasing frequency of positive patch

* Corresponding author at: KTH Royal Institute of Technology, School of Chemical Science and Engineering, Department of Chemistry, Division of Surface and Corrosion Science, SE-10044 Stockholm, Sweden. Tel.: +46 8 790 6878; fax: +46 8 208284.

E-mail addresses: yolanda@kth.se, yolanda.hedberg@ki.se (Y.S. Hedberg), carola.liden@ki.se (C. Lidén), ingero@kth.se (I. Odnevall Wallinder).

tests to Cr in Denmark since 1995–1997, significant in one study [2] but non-significant in another study [3]. Cr contact dermatitis has been associated with Cr-tanned leather and leather products since the 1950s [4,5]. More than 90% of the leather produced worldwide (approximately 2 billion m²) [6] is Cr-tanned [6–8]. Between 7 and 50% of approximately 9 500 leather products tested and reported in the literature since 2000 contain Cr(VI) in concentrations exceeding the limit of detection (3 mg/kg_{leather}) according to the ISO 17075 standard [9–13]. Cr release from leather products poses problems to Cr-allergic individuals [1,9], since such exposure may cause severe dermatitis. Cr allergy is considered to have a poor prognosis [14]. Denmark did propose to limit the amount of Cr(VI) in leather by Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) [15]. A restriction has been decided and it is anticipated to enter into force within the European Union (EU) in 2015 [16]. The limit is based on the ISO 17075 standard [17] for leather products, which determines Cr(VI) by extraction from leather powder in phosphate buffer (PB) for 3 h. The scientific literature, the ISO standard, and the restriction all focus on the release of Cr(VI) from leather without considering release of Cr(III).

Cr is both an essential element and potentially toxic to water organisms, depending on its concentration, state of oxidation, and formation of complexes [18,19]. For microorganisms and plants, it is a non-essential metal [20]. Active Cr(VI) is highly toxic due to a large ability to penetrate cell membranes [21] and is therefore generally considered more toxic than Cr(III) [20]. However, studies on fresh water algae show that Cr(III) is more toxic compared with Cr(VI) [22], and the possibility of Cr(III) genotoxicity has been emphasized [23]. Within the framework of environmental risk assessment in EU, predicted no effect concentrations of 3.4 µg/L Cr(VI) and 4.7 µg/L Cr(III) have been reported for surface water [24].

The state of oxidation and the extent of complexation of Cr in leather and upon its release into solution are, hence, essential both for ecotoxicological and human health considerations. The aim of this study was to assess potential correlations between the bulk- and surface chemistry of leather, released Cr(III) and Cr(VI), and the abilities of co-released leather-specific species to reduce and form complexes with released Cr.

2. Materials and methods

2.1. Leather

Four differently tanned, unfinished, leathers (from cattle) from the normal production were received from three European tanneries. All materials were tanned and post-tanned, but not coated and without finish (so called crust leather). Detailed descriptions on general leather processing steps, always including the tanning and post-tanning steps, are given in references [6,8]. The leather and tanning procedures are described below:

- (1) Cr^{Cr}_{gloves}: Cr-tanned followed by post-tanning using Cr, intended use in working gloves (generally low price leather);
- (2) Cr^{Cr}: Cr-tanned followed by post-tanning using Cr and synthetic tannins;
- (3) Cr^{veg}: Cr-tanned followed by post-tanning using vegetable and synthetic tannins;
- (4) Veg^{veg}: vegetable-tanned followed by post-tanning using vegetable tannins (mimosa).

The Cr^{Cr} and Cr^{veg} leather samples were selected to investigate the effect of post-tanning. The samples were from the same tannery, they had been identically treated and Cr-tanned, except for different post-tanning. The Veg^{veg} leather was investigated for comparative reasons. All leather samples represent leathers of

relevance for human and environmental exposure available on the European market. Non-coated samples were chosen to enable comparisons between Cr release and Cr oxidation state on the leather surface.

2.2. Scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy (EDS)

Surface morphology was investigated by means of SEM using a table-top SEM, Hitachi TM-1000, equipped with EDS for elemental analyses. To minimize charging effects, small (µm) pieces of leather fibers were spread out on carbon tape.

2.3. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

Chemical information of the leathers (information depth: a few µm) was obtained by ATR-FTIR using a Perkin Elmer Spectrum One instrument. Peaks of CO₂ and H₂O were automatically removed, and a background spectrum automatically subtracted from the spectra. A new background spectrum was obtained prior to the measurements or when any deviation from 100% transmittance was observed between the measurements after gentle cleaning of the crystal with ethanol and paper tissue.

2.4. X-ray photoelectron spectroscopy (XPS)

XPS was used for compositional analysis of the outermost surface of duplicate samples of all leathers before and after exposure for 3 h in artificial sweat (ASW) and PB at room temperature, respectively. After exposure, the samples were dried for 24 h at 20–25 °C (in darkness), and gently shaken (12°, bi-linearly, 22 cycles/min). Experimental details are given elsewhere [25]. Wide spectra and detailed spectra (20 eV pass energy) on Cr 2p, C 1s, N 1s, S 2p, and O 1s of each test item were run using a Kratos AXIS Ultra-DLD X-ray photoelectron spectrometer (Kratos Analytical) with a monochromatic Al X-ray source (150 W) on areas approximately sized 700 µm × 300 µm.

2.5. Exposure to artificial sweat and phosphate buffer for extraction of Cr

Triplicate samples of each leather (1 g, approx. size 3.5 cm × 3.5 cm × 0.2 cm) were exposed (extracted, immersed) at room temperature (20–25 °C) in darkness, during bilinear shaking (22 cycles/min, 12°) to 50 mL of ASW or PB for 3 h, respectively. One blank sample (without leather) was exposed in parallel for all experimental conditions. ASW (initial pH 6.5) consists of 5.0 g/L NaCl, 1.0 g/L lactic acid, and 1.0 g/L urea, adjusted to pH 6.5 ± 0.05 by NaOH. PB (initial pH 8.0) is composed of 11.8 g/L K₂HPO₄·3H₂O, adjusted to pH 8.0 ± 0.1 by phosphoric acid, and was used non-deaerated. PB is the extraction solution stipulated in the ISO 17075 standard [17], while ASW, according to EN 1811:2011 [26], is considered to be of higher relevance for skin exposure. Ultrapure water (18.2 MΩ cm) was used as solvent for all solutions, and all equipment was prior to use acid-cleaned (10% HNO₃ for at least 24 h) followed by four subsequent rinsing events using ultrapure water. The solution samples (without the leather) were centrifuged at 704 rcf after exposure (extraction), and each sample was separated into two different acid-cleaned storage flasks. One solution sample was acidified (65% ultrapure nitric acid) to a pH < 2 for total Cr analysis using flame atomic absorption spectroscopy (AAS), and the other sample was non-acidified and frozen prior to spectrophotometric analysis of Cr(VI) in solution.

2.6. Atomic absorption spectroscopy

Total released Cr concentrations in acidified solution samples were determined by means of AAS using 0 (ultrapure water), 0.5, 1.5, 5, 10, 15, and 45 mg/L Cr (in 1% HNO₃) as calibration standards. Quality controls of known concentration were measured after at least every 5th sample and the instrument was re-calibrated when the measured concentration deviated more than 10% from the nominal concentration. The limit of detection in the samples was estimated to 0.015 mg/L Cr (three times the highest standard deviation of the blank samples), and the limit of determination to 0.05 mg/L (10 times the standard deviation). All reported Cr concentrations were exceeding the limit of determination significantly.

2.7. Spectrophotometry

The Cr(VI) concentration in non-acidified solution samples (frozen prior to determination) was determined via the formation of a pink complex formed with diphenylcarbazide (DPC), spectrophotometrically measured at 540 nm. Calibration samples were prepared from the blank extraction/exposure solution (ASW or PB) and known concentrations of Cr(VI). All samples, phosphoric acid (70 vol%) and the DPC solution (1.0 g 1,5-diphenylcarbazide in 100 mL acetone acidified with one drop of glacial acetic acid) had the same volume ratio as given by the ISO 17075 standard test [17] (corresponding to 96 vol% sample, 2 vol% phosphoric acid, 2 vol% DPC solution, respectively). The calibration standards were prepared at concentrations of 0, 125, 250, 500, and 1000 µg/L Cr(VI). All calibration curves were linear ($R^2 = 0.9964$ – 0.9999). The limit of determination was estimated to approximately 60 µg/L Cr(VI). All reported values exceeded the limit of determination.

2.8. Data presentation and statistical analysis

All release data presented is normalized to the dry mass of the leather, mg/kg_{leather} (1 mg/kg_{leather} corresponds in this study to 0.02 mg/L for 1 g sample), or normalized to the geometric leather surface area, mg/cm² (1 mg/cm² corresponds in this study to 20 mg/L for 1 cm² sample), as average values of triplicate samples with the corresponding blank concentration, if positive, subtracted. The approximate thickness of the dry leather samples ranged from 1 mm (Cr^{Cr}_{gloves}) to 2.5 mm (Cr^{veg}). Blank concentrations were below the limits of determination for Cr(VI) and for total Cr. Cr(III) was determined as the total Cr concentration with any detected Cr(VI) subtracted. To identify the statistical significance of observed differences in experimental findings, a Student's *t*-test of unpaired data with unequal variance was employed between two different sets of data. Differences were counted and denoted as "significant" when $p < 0.05$, with higher significance for smaller *p*-values.

2.9. Complexation/reduction capacity of solution samples

In order to estimate the capacity of the extraction solutions to reduce released Cr(VI), extraction samples of each of the four leather types (as described in Section 2.5) were analyzed. DPC forms a colored (pink) complex with chromate (Cr(VI)). The absorbance was measured spectrophotometrically prior to the addition of DPC, in order to obtain a background value, and 2–3 min after the addition of known amounts of Cr(VI). The absorbance was also measured 45 min (instead of 2–3 min) after the addition of Cr(VI), to study equilibrium kinetics.

Table 1

Selected observed ATR-FTIR bands and assignments.

Observed bands at (cm ⁻¹)	Assigned to
3302–3313	"Peak A", stretching vibrations of the N–H of amino acids [29,31]
3077–3088	"Peak B", stretching vibrations of N–H of amino acids [29]
1633–1653	"Amide I", mainly assigned to C=O stretching vibrations of the protein backbone [29–32,34,35]
1540–1549	"Amide II", N–H bending vibrations (60%) and C–H stretching (40%) vibrations [30–32], and/or COO ⁻ antisymmetric stretch (Aspartate), and/or tryptophan (1554 cm ⁻¹) [28]
1231–234	"Amide III", C–N stretching vibrations, N–H in plane bending vibrations from amide linkages, and wagging vibrations from CH ₂ groups from the glycine backbone and proline side chains [29–32]
1450	C–H bending vibrations (aliphatic groups) [28,34]

3. Results and discussion

3.1. Bulk and surface characterization of leathers

Morphological differences of the four leathers are illustrated in Fig. 1. Cr was identified (information depth of a few µm) for all Cr-tanned leathers, and sulfur for all leathers. The EDS detector did not allow any light elemental analyses (O, C or N). No significant differences were observed in the observed Cr/(Cr + S) atomic ratio among the Cr-tanned leather samples being 56 ± 5.1 at% for Cr^{Cr}_{gloves}, 42.9 ± 2.2 at% for Cr^{Cr}, and 47.3 ± 3.6 at% for Cr^{veg}. For comparison, the ideal ratio of the basic chromium sulfate (Cr(SO₄)OH), used as tannin, is 50 at%. However, as industrial basic chromium(III)sulfate is impure, this ratio may vary [6]. A lower ratio than 50 at% may also imply the presence of sulfur originating from other leather constituents, e.g. residual sulfuric acid, an acid used in the tanning process [8], or from sulfur containing amino acids in collagen [27] or other proteins.

Fig. 2 shows ATR-FTIR spectra of the unexposed leathers. All leathers showed typical collagen absorption bands, Table 1. The amide III band (random coil) is seen at 1245 cm⁻¹ and the amide III helix at 1264 cm⁻¹ [28]. All three amide bands (I–III) are caused by the triple helix conformation [29]. The amide peak A was broadened for all leathers due to hydrogen bonds [29–32]. There are additionally two bands at 2919–2924 cm⁻¹ and at 2851–2854 cm⁻¹, possibly related to C–H stretching by fatty acids [33]. These bands may derive from fats or other treatments of the top surface of the leathers, since they were more intense at the top (smooth side) compared with the bottom (rough) sides of all leathers (Fig. S1, supplementary data). Cr^{Cr}_{gloves} did not show any of the bands related to vegetable tannins around 1160 cm⁻¹ (assigned to CH₃ antisymmetric rocking (aliphatic) and CH rocking (aromatic) vibrations) [28], or at 1000–1350 cm⁻¹ (related to bending of aromatic groups of the vegetable tannins) [31].

Cr was identified by means of XPS at the outermost surface of all Cr-tanned leathers, but not for the Veg^{veg} leather, findings in agreement with EDS. Relatively high observed binding energies of Cr, all > 577.7 eV, implied a hexavalent state of Cr [36–38], Table 2. However, as also trivalent chromium sulfate compounds show relatively high binding energies (578.4–580.8 eV – corrected to C 1s at 285.0 eV [39]), no unambiguous determination of the oxidation state of Cr could be made. While the binding energies and amounts of Cr (0.3–0.7 at%) in the outermost surface of Cr^{Cr}_{gloves} and Cr^{Cr} remained relatively constant before and after exposure to both ASW and PB, findings were different for Cr^{veg}. This leather showed two Cr 2p_{3/2} peaks centered at 578.5 eV and 581.1 eV for the unexposed state, in contrast to the other Cr-tanned leathers.

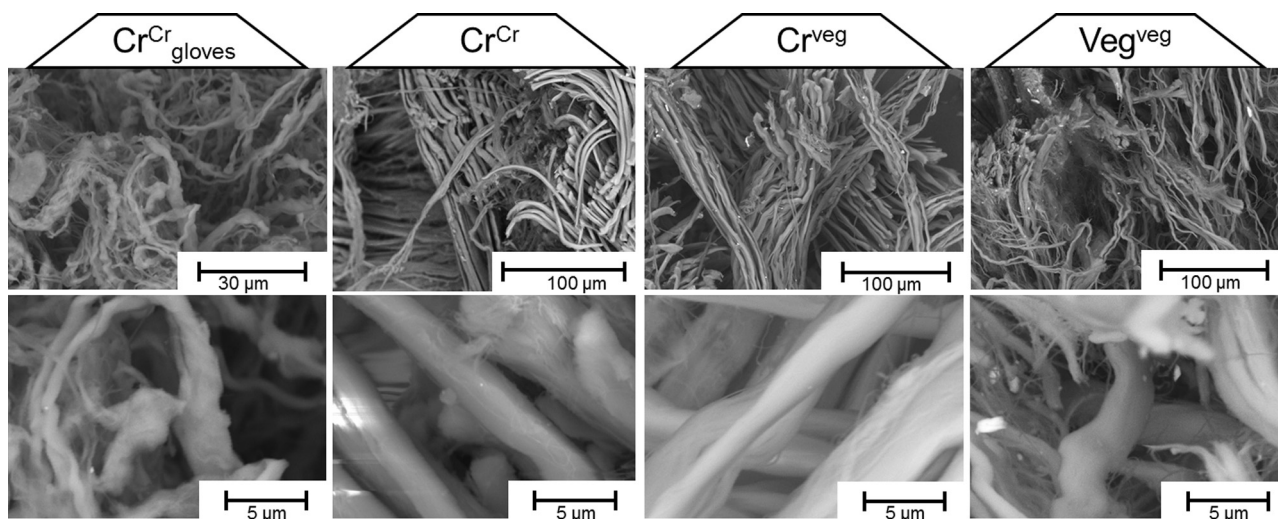


Fig. 1. SEM images of lower (top) and higher (bottom) magnification up to 10,000 $\times$.

This suggests a different chemical surrounding and bonding for Cr in Cr^{veg}, possibly connected to vegetable tannins. After exposure, no Cr was detected in the outermost surface of this leather, findings in contrast to the other Cr-tanned leathers. Sulfur was assigned as sulfate (168.5 eV [40,41]) in all leathers with a few exceptions, Table 2. One exception was one sample of Cr^{Cr} exposed to PB, for which no Cr was detected and that showed a S 2p binding energy of 164.0 eV, possibly assigned to S–S and S–H bonds in proteins [40,42,43]. Another exception was one sample of the unexposed Veg^{veg} leather, that revealed a small (0.01 at%) peak at 160.5 eV, while all other samples of this unexposed or exposed leather only showed sulfate peaks (most probably due to residuals of sulfuric acid used in the tanning process [8]). The relative surface atomic ratio of Cr/(Cr + S) was 38–45% for the Cr^{Cr} gloves leather (unexposed and exposed to PB) and the Cr^{Cr} leather (unexposed), in agreement with EDS findings, but significantly higher for the unexposed Cr^{veg} leather (99 at%), and lower for the exposed Cr^{Cr} leather (5–22 at%) and Cr^{Cr} gloves leather exposed to ASW (22 at%). The high ratio observed for the Cr^{veg} leather suggests, in addition to the different Cr binding energies for the Cr^{veg} leather compared with the other Cr-tanned leathers, that Cr at the surface is bound to other species than sulfate, most probably vegetable tannins. The lower

ratios observed after exposure for the Cr^{Cr} gloves and Cr^{Cr} leathers may indicate that Cr was preferentially released compared with sulfuric acid residuals.

No significant difference in XPS binding energies for oxidized carbon (C 1s) or its relative proportions were observed among the different samples and exposure conditions. The C1s peak at 285.0 eV (61 at% of total C, ranging from 30 to 74 at%) was assigned to C–C and C–H bonds [32,44], the peak at 286.2 $\pm$ 0.3 eV (21 at%, 15–27 at%) to C α (peptidic residues), C–O, and C–N bonds [32,44], the peak at 287.7 $\pm$ 0.7 eV (10 at%, 6–17 at%) to N–C=O bonds [32], and the peak at 289.0 $\pm$ 0.8 eV (10 at%, 4–25 at%) to O–C=O bonds [32]. Three O 1s peaks were observed, the first at 531.8 $\pm$ 1.1 eV, mainly assigned to O=C in peptidic carbonyl groups [32], the second at 533.2 $\pm$ 1.0 eV to C–OH bonds [32], and the third peak (only for unexposed Veg^{veg} and Cr^{veg}) at 536.5 $\pm$ 1.0 eV, most probably assigned to polyphenols in the vegetable tannins [45,46]. After exposure, no oxygen peak corresponding to the polyphenols was observed, neither in Veg^{veg} nor Cr^{veg}. This observation correlates with the lack of Cr at the surface of Cr^{veg} after exposure. Cr could hence partially or completely be bonded to vegetable tannins on the unexposed surface of Cr^{veg}, supported by its higher binding energy compared with the other Cr-tanned leathers.

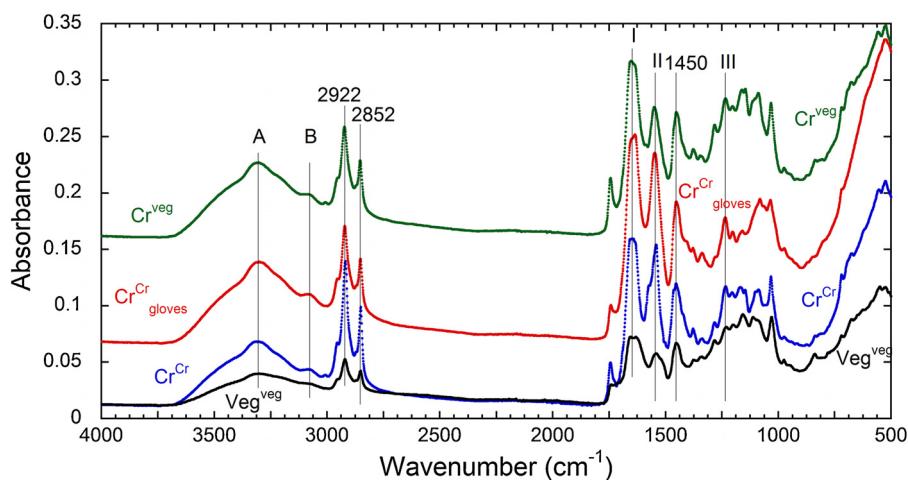


Fig. 2. ATR-FTIR average spectra (each based on 25 single spectra) collected for the top (smooth side) surface of the four unexposed leathers. The spectra are off-set for clarity. (For interpretation of the references to color near the citation of this figure, the reader is referred to the web version of the article.)

Table 2

XPS binding energies (eV), normalized to the C 1s peak at 285.0 eV, of Cr 2p_{3/2} and S 2p for unexposed, and exposed leathers (to ASW and PB for 3 h at room temperature, respectively), presented as average values and standard deviation between two different, independent, samples.

Leather	Exposure	Cr 2p _{3/2}	S 2p
Cr ^{Cr} _{gloves}	Unexposed	578.1 ± 0.02	168.4 ± 0.8
	ASW	577.7 ± 0.02	168.9 ± 0.6
	PB	578.0 ± 0.1	168.5 ± 0.1
Cr ^{Cr}	Unexposed	578.0 ± 0.01	168.0 ± 0.6
	ASW	578.1 ± 0.09	168.5 ± 0.02
	PB	578.5 ^a	164.0; 168.6 ^b
Cr ^{veg}	Unexposed	579.7 ± 1.6	168.2 ± 0.06
	ASW	N/D	168.6 ± 0.05
	PB	N/D	168.2 ± 0.2
Veg ^{veg}	Unexposed	N/D	160.5; 168.3 ^b
	ASW	N/D	168.6 ± 0.002
	PB	N/D	168.8 ± 0.3

N/D – not detected.

^a only detected for one sample.

^b different binding energies observed for duplicate samples.

The presence of Cr(VI) in leather can qualitatively be assessed by adding a drop of DPC on the surface and observing the formation of a visible red-violet complex [5]. The natural pH of leather is 4.4 [47] and the DPC solution is acidified with acetic acid (1 drop to 100 mL). DPC forms a colored complex with Cr(VI) (dichromate) at acidic conditions [48], but not with Cr(III). However, DPC can be oxidized to diphenylcarbazone at alkaline conditions and in the presence of oxygen [48], which can form the same colored complex with Cr(III) as DPC forms with Cr(VI) [48]. DPC drops were applied on all unexposed leather samples. After 5 min, a weak red-violet color was visible for the Cr^{Cr}_{gloves} leather, while it was not clearly visible for the other (colored) leathers, Fig. S2 (supplementary material). This color was very strong for the Cr^{Cr}_{gloves} leather after several days and also visible for Cr^{Cr} and Cr^{veg} leathers, but not for the reference Veg^{veg} (as expected), Fig. S2. While it is unambiguously evident that some Cr(VI) is present in the Cr^{Cr}_{gloves} leather due to the fast color reaction with DPC, it is most probably also the case for the other two Cr-tanned leathers, but cannot unambiguously be concluded.

3.2. Cr(III) and Cr(VI) release into ASW and PB

Fig. 3 shows the total amount of Cr (Fig. 3a), Cr(III) and Cr(VI) (Fig. 3b) released from each leather into ASW (initial pH 6.5) and PB (initial pH 8.0), respectively, after 3 h of exposure. Corresponding pH changes are presented for each solution (Fig. 3c). In general, the total amount of released Cr in solution was relatively similar (Fig. 3a and b) despite large differences in solution pH, Fig. 3c. One of the Cr-tanned samples (Cr^{Cr}_{gloves}) released less total Cr in PB compared with ASW (non-significantly when normalized on the surface area (Fig. 3a), but significantly, $p < 0.05$, when normalized to dry mass (Fig. S3, supplementary data)). The opposite situation was observed for the other two Cr-tanned samples with higher release in PB compared with ASW (significantly, $p < 0.05$, when normalized to the surface area, Fig. 3a, and significantly, $p < 0.01$, only for Cr^{Cr} when normalized to dry mass, Fig. S3). Cr was predominantly released as Cr(III) for all investigated leather samples. Cr^{Cr}_{gloves} was the only leather that released Cr(VI) in PB. ASW showed a significantly lower buffer capacity compared with PB, which resulted in a large pH reduction for all leather samples (Fig. 3c), whereas PB maintained a stable pH above 7.5.

It was clearly shown that Cr(III) was the predominantly released species from different types of Cr-tanned leather samples, findings in agreement with literature observations for Cr-tanned leather

[10,11,49–51]. It is considered less likely that Cr(VI) is released in sweat and under prolonged skin contact, unless the leather is exposed to a very dry environment, UV irradiation, and/or oxidizing or alkaline species [50,52–60]. The amount of Cr(VI) released from Cr^{Cr}_{gloves} (at most 26.1 mg/kg_{leather}) in the present study is among the higher values reported in the literature for leather products (<1–62 mg/kg_{leather}) [9–12] and for non-finished leathers elicited to form Cr(VI) (up to 96 mg/kg_{leather}) [53]. Despite this high amount in the leather was no Cr(VI) released in ASW, consistent with previous studies [5,47]. The effect of vegetable post-tannins on the Cr(VI) content in leather has been largely investigated, since most vegetable post-tannins reduce the Cr(VI) content efficiently [50,53–55,59,61,62]. The effect of vegetable post-tannins on the release of Cr(VI) was not investigated in this study, since no measurable amounts of Cr(VI) were present in solution either from the Cr^{Cr} or the Cr^{veg} samples at any conditions.

3.3. Reduction/complexation capacity of extraction solutions

Fig. 4 shows changes in absorbance (spectrophotometrically measured at 540 nm) upon the addition of Cr(VI) in extraction solutions from all four investigated leather samples. A lower slope compared to the reference sample (the solution without leather extracts) corresponds to a higher reduction and/or complexation capacity of the sample, which means that added Cr(VI) is reduced by the solution. The extraction sample for Cr^{Cr}_{gloves} showed no reduction/complexation capacity. The Cr^{Cr} and Cr^{veg} leather samples showed significant reduction/complexation capacities in both solutions, findings that may explain the lack of Cr(VI) in solution from these leathers and minor or no presence of Cr(VI) at their surfaces. Since the Cr^{Cr}_{gloves} extraction solution was uncolored, the absorbance value was initially close to 0, while both samples Cr^{Cr} and Cr^{veg} were colored before adding DPC to both solutions. After adding DPC, the absorbance increased significantly only for Cr^{Cr}_{gloves} among the Cr-tanned samples due to the presence of Cr(VI) in that extraction solution. Measurements 2–3 min and 45 min after addition of Cr(VI) did not differ significantly for the Cr-tanned solution samples. It can therefore be stated that the surface of Cr^{Cr}_{gloves} contained Cr(VI) and that Cr(VI) was observed in PB due to its low reduction/complexation capacity. The Veg^{veg} samples were also colored initially in both solutions and revealed an increase in absorbance after addition of DPC. This effect was not attributed to the pink color of the Cr(VI)-DPC complex but related to visible red particles formed after the addition of DPC. The presence of these red particles and their dispersion properties largely influenced the absorbance (fluctuations) in PB (Fig. 4b). Precipitation of these particles occurred with time, seen as a strong reduction in absorbance after 45 min (Fig. 4b). Interferences by the formation and the presence of red particles from mimosa (the vegetable tannin of the Veg^{veg} leather) and DPC in the solution have been reported previously [54].

3.4. Correlation between bulk- and surface chemistry of Cr-tanned leather and the release of Cr(III) and Cr(VI)

A strong correlation was evident between the oxidation state of released Cr and the solution complexation/reduction capacity containing released leather species. This is expected, since even if Cr(VI) would be formed at dry conditions at the leather surface in contact with air [53,57], it would be reduced in the presence of co-released reducing agents. It is however unclear if the reducing agents or Cr are released at a higher rate upon long-term exposure, or if partially or completely depleted. This requires further investigations. There might be a risk of an increased Cr(VI) release as a consequence of co-release of reducing agents from the leather,

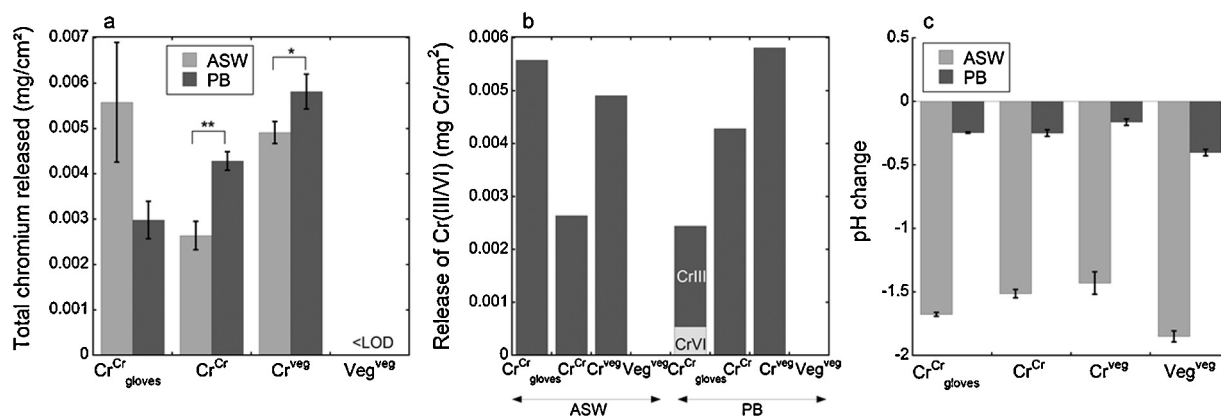


Fig. 3. Released amounts of total Cr (a), Cr(III) and Cr(VI) (b) in PB (initial pH 8.0) and ASW (initial pH 6.5) after 3 h of exposure at 20–25 °C normalized on surface area, with corresponding pH changes (c). The error bars show the standard deviation between triplicate samples. Released amounts of total Cr were below the limit of detection (<LOD) for the Veg^{veg}, and of Cr(VI) for Cr^{Cr}, Cr^{veg}, and Veg^{veg} in PB, and all samples in ASW. Corresponding released amounts of total Cr normalized on dry leather mass are shown in Fig. S3 (supplementary data). The asterisks indicate significant (* $p < 0.05$; ** $p < 0.01$) differences.

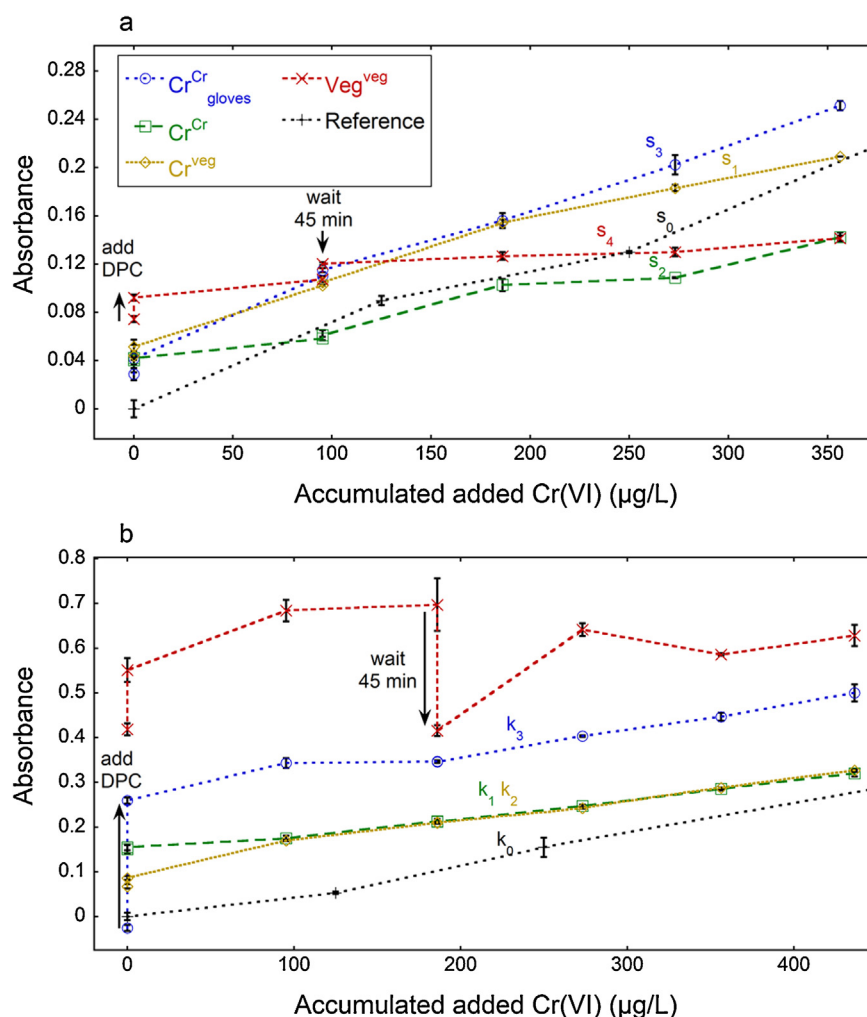


Fig. 4. Absorbance of different extraction samples (3 h, 20–25 °C) of ASW (a) and PB (b) in dependence of added DPC solution, added Cr(VI) and waiting time (before measurement). A significantly lower slope compared with the reference slope ($s_0, k_0 = 0.00061\text{--}0.00065$) corresponds to a higher reduction capacity of the sample. The slopes s and k in (a) and (b), respectively, are $s_0 \approx s_3$ ($0.00059\text{--}0.00061$) $> s_1$ (0.00046) $> s_2$ (0.00028) $> s_4$ (0.00016) and $k_0 \approx k_3$ ($0.00062\text{--}0.00065$) $> k_1 \approx k_2$ ($0.00043\text{--}0.00046$). The dotted lines are only guidance for the eye. (For interpretation of the references to color near the citation of this figure, the reader is referred to the web version of the article.)

since a prolonged washing step (removal of formic acid residuals) in the tanning process has been reported to increase the amount of extracted Cr(VI) [53].

While released Cr(VI) always is present as negatively charged chromate in solution, the form of released Cr(III) most probably depends on the leather type. This was evident in this study for the Cr^{veg} sample (surface Cr bound to polyphenols) compared with the Cr^{Cr} and Cr^{gloves} samples (surface Cr bound to sulfate). The chemical form of Cr is important for diffusion rates in human skin and for Cr dermatitis [4,63–67]. The chemical form is influenced by different leather species, and also by the solution pH [47]. The chemical form of Cr at the leather surface and its released chemical form will furthermore influence its release kinetics and dependence on pH and solution. Further studies are needed to investigate the solution and time dependence of Cr release from differently tanned leathers.

4. Conclusions and future perspectives

Co-released reducing/complexing leather-specific species were able to reduce released Cr(VI) from Cr-tanned leather, both in ASW (pH 4.7–6.5) and in PB (pH 7.5–8.0). This reduction capacity correlated with findings of the surface presence of Cr and released Cr, that is, an increased capacity results in a reduced Cr(VI) release and/or the presence at the surface. Leather without this capacity and with fewer aromatic groups, possible to identify using ATR-FTIR, revealed Cr(VI) both at the surface and in PB. Cr was only released as Cr(III) in both solutions for Cr-tanned leathers of high reducing/complexation capacity. It was however not evident whether Cr was present in its tri- or hexavalent state at their surfaces. No Cr(VI) was measured in the acidic ASW solution for any of the investigated leathers.

Cr(VI) in leather has been restricted in the EU by REACH that limits Cr(VI) in consumer and occupational leather products. Control of compliance shall be done according to the ISO 17075 standard. Since the leathers are tested only once and the formation and release of Cr(VI) and Cr(III) may be dependent on time and several environmental factors, it is considered of large interest to investigate the effect of relevant exposure parameters on the Cr(III) and Cr(VI) release from Cr-tanned leather. Investigations on the effect of exposure time, deaeration, experimental conditions, temperature, surface area, relative humidity, and repeated exposure, are ongoing by the authors. Further studies should also investigate the amount of Cr deposited on the skin from Cr-tanned leather, and how the amount and the speciation of released Cr from leather affects their ability to cause skin eczema in already Cr-allergic individuals.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jhazmat.2014.08.061>.

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Supplementary data

Correlation between bulk- and surface chemistry of Cr-tanned leather and the release of Cr(III) and Cr(VI)

Yolanda S. Hedberg^{a,b,*}, Carola Lidén^b, Inger Odnevall Wallinder^a

^aKTH Royal Institute of Technology, School of Chemical Science and Engineering, Department of Chemistry, Division of Surface and Corrosion Science, SE-10044 Stockholm, Sweden

^bInstitute of Environmental Medicine, Karolinska Institutet, Box 210, SE-17177 Stockholm, Sweden

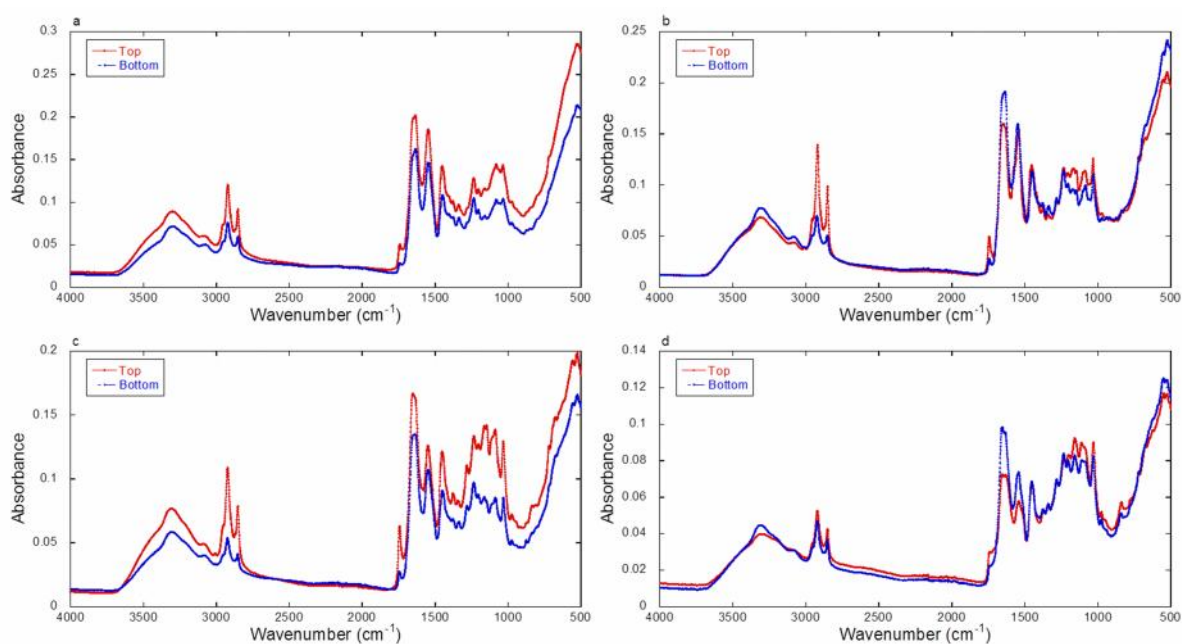


Figure S1. ATR-FTIR average spectra (each based on 25 single spectra) for the top (smooth) and bottom (rough) side of Cr^{Cr}_{gloves} (a), Cr^{Cr} (b), Cr^{veg} (c), and Veg^{veg} (d), respectively.

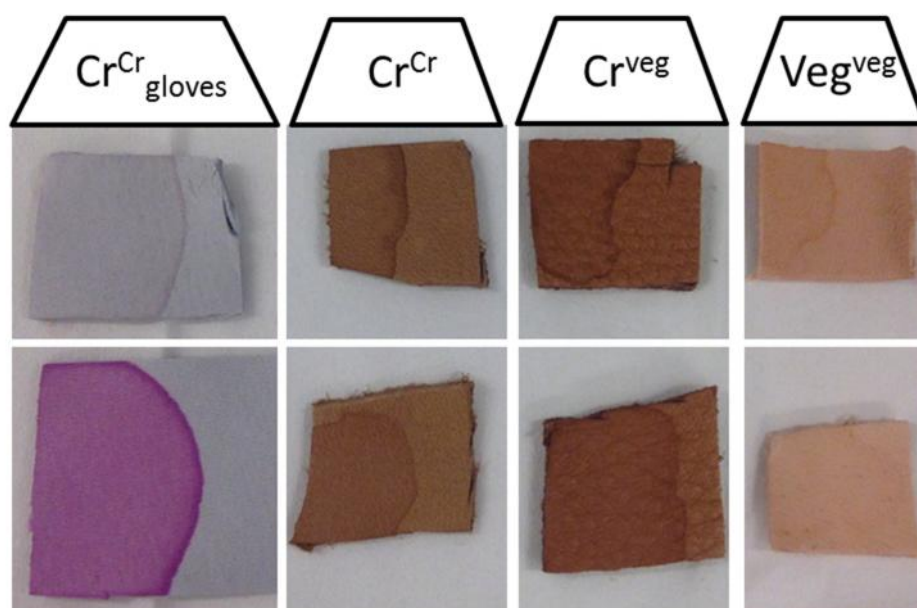


Figure S2. Unexposed leathers 5 min (top) and 3-7 days (bottom) after application of a drop (approx. 500 μ L) of DPC solution. A pink color implies the presence of Cr(VI). All leathers are shown from the top (smooth) side.

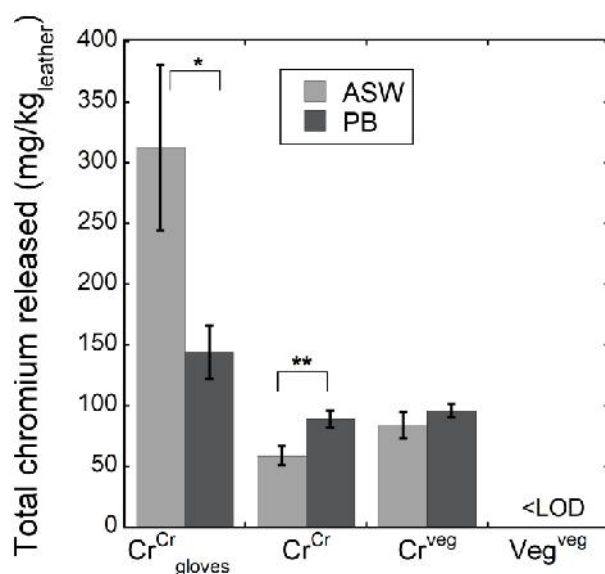


Figure S3. Released amounts of total chromium in PB (initial pH 8.0) and ASW (initial pH 6.5) after 3 h of exposure at 20-25 °C normalized on surface area. The error bars show the standard deviation between triplicate samples. Released amounts of total Cr were below the limit of detection (<LOD) for Veg^{veg}. The asterisks indicate significant (* $p < 0.05$; ** $p < 0.01$) differences.