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The ability of sweat and buffer solutions to reduce hexavalent chromium of relevance for leather extraction

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ABSTRACT

The European Union restricted the amount of hexavalent chromium (Cr(VI)) in leather in 2015, but skin allergy cases due to Cr-tanned leather are not declining. Standardized extraction methods have been criticized to both over- and underestimate the expected amount of bioavailable Cr(VI) in leather. This study aims to evaluate the ability of four extraction solutions to reduce or preserve Cr(VI): artificial sweat solutions (ASWs) of pH 4.7, 6.5, and 8.0, and phosphate buffer (PB) of pH 8.0. This was investigated by incubating each solution with added Cr (VI) as a function of time, and then measuring the recovered Cr(VI). All solutions, especially PB, preserved Cr(VI) for 24 h. These solutions were also pre-exposed to Cr-free vegetable-tanned leather (VTL) before incubation with Cr(VI). Released vegetable tannin species strongly reduced Cr(VI), with up to 4000 μg/L added Cr(VI) reduced in all solutions after 24 h. However, after 1 h, Cr(VI) was still detectable in extraction solutions at pH 6.5 and above. The reduction of Cr(VI) in relevant extraction solutions is hence a process dependent on time, pH, and the presence of co-released leather species. All extraction solutions, but least PB, have the potential to underestimate any Cr(VI) present on the surface of leather.

1. Introduction

A significant portion of the general population is affected by metal allergies. However, few know about chromium-associated allergies and are unaware that they may be exposed to chromium (Cr) daily (Thyssen and Menné, 2010). It has been reported that 1% of the general population is allergic to Cr (Hedberg, 2020), with Cr-related contact dermatitis cases having been reported since 1908 (Thyssen and Menné, 2010). Cases due to repetitive contact with chrome-tanned leather have recently risen in proportion (Alinaghi et al., 2019). The European Union (EU) 2015-enforced limit of 3 mg hexavalent chromium (Cr(VI)) per kg leather has so far not lowered the number of contact dermatitis cases due to Cr-tanned leather reported in Denmark (Alinaghi et al., 2021). As a result, the European Chemicals Agency (ECHA) suggested in 2021 to lower the EU-enforced limit of Cr(VI) from 3 to 1 mg/kg, to be mandated via the REACH regulations within 5 years (ECHA, 2020).

When in contact with dermal skin, Cr(VI) on the surface of leather has the potential to be absorbed by the skin and cause allergic reactions or other adverse health effects. As one of the most rapidly diffusing chemicals, Cr(VI), as negatively charged chromate, can penetrate the skin efficiently at rates similar to water and about 10,000 times faster than Cr(III) (Van Lierde et al., 2005a; Gammelgaard et al., 1992; Spruit and van Neer, 1966; Samitz et al., 1967). This means that any time-dependent reduction of Cr occurring in sweat competes with the rapid mobilization of chromates through the skin. The major form present in, and released from, leather is Cr(III). However, the oxidation of Cr(III) to Cr(VI) on the surface of leather does occur under certain conditions, such as at low humidity in the presence of oxygen and absence of reducing agents (Mathiason et al., 2015).

In contrast to Cr(III), which can form both anions and cations in aqueous (water) solutions, the only relevant forms of Cr(VI) are mono- or dichromates, which are negatively charged ions and do not form complexes with organic ligands, such as proteins, and therefore possess a higher skin permeability than Cr(III) species (Hedberg, 2018).

To determine the amount of Cr(VI) available on the surface of leathers, extraction tests are used; however, these come with limitations. For example, extraction solutions can reduce Cr(VI) to Cr(III), which may skew the measured amount of Cr(VI). Many artificial sweat solutions (AWSs) exist for detecting metal release in a biologically relevant manner (Midander et al., 2016). Some of these can be expected to have

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some reduction capacity towards Cr(VI) due to their weakly acidic pH or the presence of reducing biomolecules. A German study (BGFA, 2003) found that the sweat of 10 investigated people had a pH range of 5.2–5.7. They then noted that the extractable Cr(VI) from leather samples was at most 30% in pH 5.5 ASW compared with a phosphate buffer at pH 8.0. They further found that the initially bioavailable Cr(VI) decreased with time in the pH 5.5 ASW.

A deaerated phosphate buffer solution with high ionic strength and buffer capacity has been particularly developed to preserve Cr(VI) extracted from leather (ISO, 2017). The same solution has been criticized as being irrelevant for skin exposure and for its ability to oxidize Cr (III) to Cr(VI) in the presence of oxidizing species during extraction (Pastore et al., 2004). Besides actual relevance for skin exposure, extraction solutions should ideally not change the oxidation state of extracted chromium from the test item (leather). This is however difficult to obtain under all conditions, as leather contains possibly reducing and oxidizing agents, as well as high amounts of acids. It is therefore of the utmost importance that experimental studies, such as this, evaluate the discrepancies between extraction solutions in terms of their ability to preserve or reduce Cr(VI). This is necessary to design tests that will best extract Cr in its original form and therefore accurately quantify bioavailable Cr(VI). This study aims to assess the Cr(VI) reduction capacities of AWSs of pH 4.7, 6.5, and 8.0, as well as a phosphate buffer solution of pH 8.0, in the presence and absence of typical co-extracted leather species.

2. Material and methods

All glassware was acid-washed in 10% nitric acid for 24 h and rinsed 4 times with ultrapure water.

2.1. Chemicals

Ammonium chloride, DL-lactic acid (90%), L-Histidine (99%), and phosphoric acid (99.99%), were obtained from Sigma Aldrich, Canada. A 10 mg/L Cr(VI) and a 999 mg/L Cr(VI) standard, both in water, were obtained from Delta Scientific Laboratory Products, Canada. Potassium hydrogen phosphate trihydrate, 99%, sodium hydroxide, sodium phosphate (dibasic, anhydrous), urea, and sodium chloride were obtained from Fisher Scientific, Canada. All chemicals had analytical grade purity.

2.2. Leather

The chromium-free vegetable-tanned leather (VTL) was tanned and post-tanned with vegetable tannins (mimosa). The VTL was not dried, surface coated, or spray dyed. For more details, please see (Hedberg et al., 2014), in which the sample is referred to as Veg^{veg}.

2.3. Preparation of diphenylcarbazide solution (DPC)

A DPC solution was prepared in accordance with ISO 17075–1:2017 (ISO, 2017). In 10 mL of acetone 99.9% (Fisher Scientific, USA), 0.1 g of 1,5-diphenylcarbazide (Sigma Aldrich, Canada) was dissolved and acidified with 5 μ L of glacial acetic acid 99.99% (Sigma Aldrich, Canada). The solution was kept in an opaque glass vial and stored in the refrigerator at 4 °C until use, with replacement every 2 weeks.

2.4. Preparation of test solutions

All solutions were prepared fresh as necessary; if this was not possible, solutions were stored in the refrigerator at 4 °C for use up to 3 days. After preparing each solution, the pH was adjusted with NaOH (in the case of the ASWs) and phosphoric acid (in the case of PB). The pH was verified using a calibrated VWR symphony B10P pH meter (VWR, Canada). The chemical compositions of all test solutions are specified in Table 1.

Table 1
Components of solutions.

Chemical (g/L)	ASW pH 4.7	ASW pH 6.5	ASW pH 8.0	PB pH 8.0
Sodium chloride	20.0	5.0	5.0	
Lactic acid	15.0	1.0	–	
Urea	5.0	1.0	–	
Acetic acid	2.5	–	–	
Ammonium chloride	17.5	–	–	
Sodium phosphate (dibasic, anhydrous)			1.98	
L-histidine			0.5	
Potassium hydrogen phosphate trihydrate				22.8

2.4.1. Preparation of ASW solutions

The pH 4.7 ASW was prepared according to NIHS 96–10 from the Swiss Watch Industry Specification (Wainman et al., 1994). The pH 6.5 ASW was prepared according to EN 1811:2011+A1:2015 (CEN, 2015). The pH 8.0 ASW was prepared according to ISO 105-E04; however, Pro-Clean was omitted (ISO, 2013).

2.4.2. Preparation of phosphate buffer pH 8.0

The solution was prepared in accordance with ISO 17075–1:2017 (ISO, 2017). The solution was deaerated with nitrogen gas (Linde, Canada) for 10 min immediately prior to usage.

2.5. Assessment of reduction capacity as a function of time and leather extracts

At least three independent experiments for each procedure were run. Each trial contained a corresponding blank solution (ASW or PB without any leather or Cr(VI) added) that was subjected to the same experimental conditions as the test samples.

2.5.1. Preparation of Cr(VI) standards in test solutions without leather

For each test solution, 3–5 standard calibration curves were generated using a 10 mg/L Cr(VI) standard to obtain the following approximate 10 mL standard concentrations: 60, 90, 125, 250, 500, 1000 μ g/L. A Mettler Toledo analytical balance was used to record the weight of the added Cr(VI) and the final volume; if this was not possible (*i.e.* for the pH 4.7 ASW first replicate, a volumetric approach (micropipettes) was used). Concentrations were then calculated using Equation (1), where V_1 and c_{stock} are the volume corresponding to the mass or volume of added Cr(VI) stock solution and its concentration, respectively, and V_2 is the final volume:

$$\frac{c_{stock} \times V_1}{V_2} = Cr(VI)\ concentration\ of\ standard \quad (1)$$

Immediately after preparation, the samples (t = 0) were analyzed in the UV–Vis spectrometer (Cary 8454, Agilent) as specified in section 2.6.

The solutions were further measured after 24 h of incubation (t = 24) at 30 ± 0.1 °C without agitation (CEN, 2015), as this is a typical maximum wait time for leather samples in extraction solution before analysis.

2.5.2. Preparation of test solutions with VTL

2.5.2.1. Preparation of leather. The leather pieces were stored in sterile plastic bags at room temperature prior to usage. Leather samples were cut into square pieces with dimensions of 1.5 cm by 1.5 cm, giving a total surface area of 4.5 cm^2. Scissors and a caliper, cleaned with ethanol (Commercial Alcohols, Canada) before cutting each sample, were used to measure and cut the VTL samples, respectively. The pieces were

weighed on an analytical balance and stored in individual sterile plastic bags at room temperature until needed or placed directly into the oven. All leather samples were handled with tweezers that had been rinsed with 1% nitric acid (65%, Sigma Aldrich, Canada).

The pre-cut leather samples were placed onto acid-washed petri dishes (propped up to expose both faces) in the oven (Isotemp, Fisher Scientific) for an exposure time of 24 h at 80 ± 1 °C. This procedure is the suggested pre-procedure (ISO 10195) for conditioning leather samples to be tested for Cr(VI) (ISO, 2018). While this leather was a Cr-free leather, we followed the same procedure to have a similar pre-conditioning as for Cr-containing leathers.

2.5.2.2. Preparation of solutions that were pre-exposed to VTL. After 24 h of oven-treatment, the leather samples were removed from the oven and immediately placed in 10 mL of test solution. The samples were then incubated (incubating rocker, VWR, or Isotemp, Fisher Scientific) for 24 h at 30 ± 0.1 °C without agitation (with the exception of the pH 6.5 samples, which were subjected to very minimal agitation due to sharing of equipment).

After incubation, the leather pieces were removed from the solutions (now termed the 'pre-exposed solutions') and filtered. The t = 0 pre-exposed solutions for the pH 4.7 and pH 6.5 ASWs were filtered twice with coarse filter paper after incubation to remove some of the color caused by the species released from the VTL. Filtering with activated carbon filters (Hztyyier) was attempted for some of the ASWs of pH 4.7 to see if it would remove the colored species completely, but it made no difference in the resulting UV–Vis spectra. To minimize the amount of time that the pre-exposed solutions were outside of the incubator, the t = 1 and t = 24 pre-exposed standard solutions were not filtered, as those were less colored compared to the pH 8.0 solutions, which were syringe-filtered immediately prior to measurement (described in section 2.6).

Appropriate volumes of the 10 and 999 mg/L Cr(VI) standard solutions were immediately added, as described in section 2.5.1, to pre-exposed solutions to obtain varying concentrations up to 4 mg/L Cr (VI). These solutions were then incubated for 1 or 24 h at 30 ± 0.1 °C.

The experimental procedures are illustrated in Fig. 1.

2.6. Spectrophotometry

All UV–vis samples were prepared according to the same volume ratio as specified in ISO 17075–1:2017; 960 μL test solution, 20 μL DPC solution, and 20 μL 70% phosphoric acid (ISO, 2017). After the addition of DPC and phosphoric acid solution, the samples were left for 10–12 min with some mechanical shaking. Samples were mixed with pipette prior to UV–Vis analysis.

Leather pre-exposed solutions, which contained some visible colour, were also measured without DPC to investigate any interferences. No interferences were observed in the wavelength range of interest (500–600 nm). Both leather pre-exposed pH 8.0 solutions (ASW and PB) had to be pushed through coarse filter paper-filled syringes 10–12 min after the addition of DPC and phosphoric acid to remove the red particulate matter formed after reaction with the VTL released species (previously described in Hedberg et al., 2014). In this case, 2 mL UV–Vis samples were prepared, keeping the same ratio of components as noted above, as some sample was absorbed into the filter paper.

Limit of detection (LOD) values were estimated for each test solution with and without pre-exposure to VTL. The LODs for the pH 4.7 ASW, pH 6.5 ASW, pH 8.0 ASW, and PB pH 8.0 without pre-exposure to leather were 46, 4, 17, and 13 μ g/L Cr(VI), respectively. For the pre-exposed solutions, the LOD increased because the baseline was tilted. The LODs for these solutions were estimated to be 64, 6, 99, and 105 μ g/L Cr (VI), respectively. The LOD values were estimated by calculating the standard deviation of the absorbance of the blanks, multiplying by 3, and then converting to concentration using the average standard calibration curve for each test solution. For calibration curves with a positive Y-intercept, the intercept value was added to blank standard deviation which had been multiplied by 3. All reported values exceed the LOD. The LODs were also qualitatively verified with the

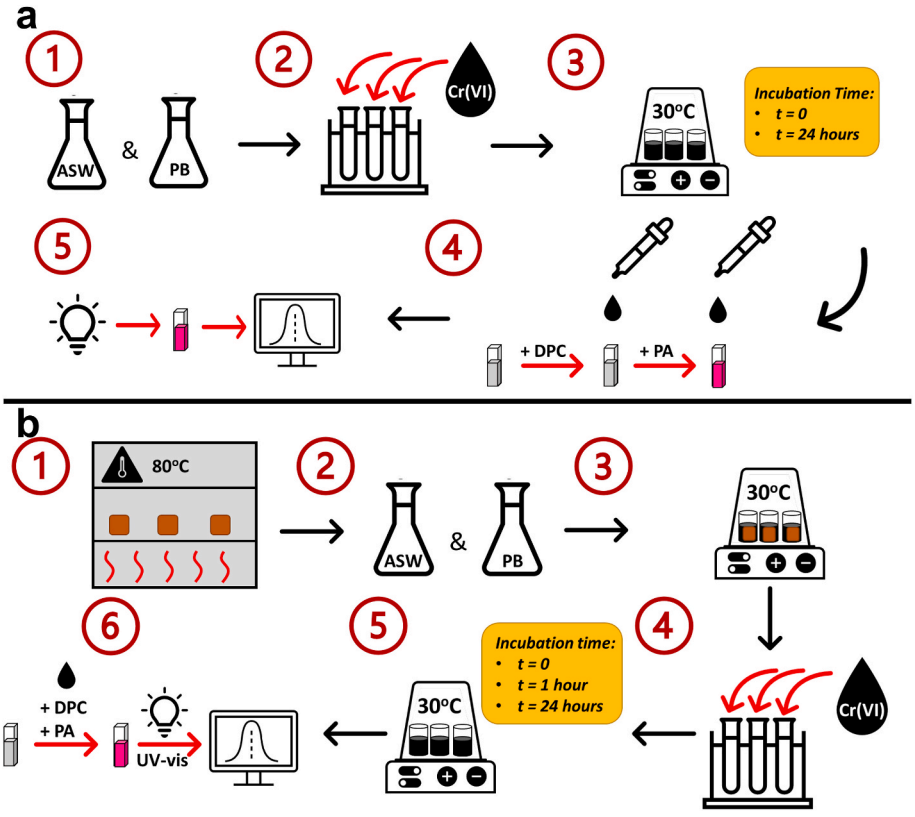

Fig. 1. (a) Experimental procedure in the absence of leather: 1) solution preparation, 2) addition of Cr(VI), 3) incubation for different durations, 4) preparation of colorimetric test, and 5) UV–Vis spectroscopic measurement. (b) Experimental procedure for pre-exposure to Cr-free VTL: 1) Conditioning of leather pieces, 2) preparation of solutions, 3) incubation of solutions with leather pieces (24 h), 4) addition of Cr (VI) to the solutions after removal of leather pieces, 5) incubation for different durations, and 6) colorimetric determination of Cr(VI). DPC – 1,5-diphenylcarbazide; PA – phosphoric acid; UV–vis – ultraviolet visible spectrophotometry; ASW – artificial sweat; PB – phosphate buffer.

spectroscopic raw data, where a value above the LOD showed a clear peak around 540 nm, distinct from the baseline noise and the spectrum of the blank.

2.7. Data presentation and analysis

The raw spectroscopic data for each sample was plotted in Origin. A straight-line baseline was selected for each sample that connected the beginning and end of each peak. The linear equation for each baseline was recorded and used to calculate the absorbance of the baseline at 546 nm (the position where the maximum of the peaks was observed for most measurements). This value was subtracted from the absorbance of the peak at 546 nm. The blank peak height, if positive, was subtracted from each calculated peak height.

These obtained peak heights were then plotted for each solution versus the originally added Cr(VI) concentrations, with the t = 0 immediate measurements, without any co-released leather species, serving as reference calibration curves.

3. Results

3.1. UV–vis spectra and their evaluation

Representative UV–Vis spectra for standard solutions initially spiked with Cr(VI) (t = 0) without pre-exposure to leather and with pre-exposure to the VTL for 24 h are displayed in Fig. 2. The Cr(VI)-related peak was easily distinguishable around 540 nm for all test solutions without leather, and each peak clearly increased in magnitude as the amount of added Cr(VI) increased. The peaks were also clearly distinguishable for the solutions pre-exposed to VTL; however, they did not always linearly correlate with the added Cr(VI) amounts, which is most obvious for the pH 4.7 ASW + VTL solution. The pre-exposed solutions also show significantly lower Cr(VI) peaks for the same amounts of added Cr(VI), further discussed below. The baseline of the spectra for the leather pre-exposed solutions was tilted as compared to the spectra of the solutions without any exposure to leather (Fig. 2). This is caused by released species, such as polyphenols (Hedberg et al., 2014), leaching from the Cr-free leather. A small peak at 450 nm was also observed for all solutions. Due to the influence of this small peak and the tilted baseline for the pre-exposed solutions spectra, the peak height at the maximum peak (546 nm) was used to evaluate all cases instead of the peak area.

3.2. Derivation of calibration curves in the different solutions

Average standard calibration curves for each test solution, without any pre-exposure to leather and immediately measured after Cr(VI) addition, are displayed in Fig. 3, and fit linearly. It was found that there was a higher variability between trials for the pH 4.7 ASW and the pH 8.0 PB (with average R^2 values of 0.955 and 0.952) as compared to the ASWs at pH 6.5 and 8.0 (with R^2 values of 0.997 and 0.996), which overlapped almost exactly with each other. The average calibration curves (of measurements immediately after Cr(VI) addition) were used as a reference (assuming 0% reduction of added Cr(VI)) to convert absorbance into measured Cr(VI) concentration for subsequent time points in corresponding solutions and after exposure to VTL. This procedure accounted for uncertainties among replicate measurements.

It is surprising that the pH 8.0 PB has a similar slope to the pH 4.7 ASW in Fig. 3, particularly because the pH 8.0 ASW slope is much greater. This may be due to different transparency/turbidity values.

3.3. Reduction capacity

Fig. 4(a–d) shows the remaining Cr(VI) in each solution as a function of time after addition of Cr(VI) versus pre-exposure to VTL (Cr-free) and subsequent addition of Cr(VI). The pre-exposed pH 4.7 ASW at t = 0 shows large reduction and high variability. The large reduction capacity is further exemplified after 1 h, as all added Cr(VI) up to 4000 μ g/L was reduced (Fig. 4a). Without exposure to VTL, this ASW had already shown about 23% reduction after 24 h incubation in comparison to the initial calibration curve at t = 0, proving to have the most effective reduction ability out of all exposure solutions.

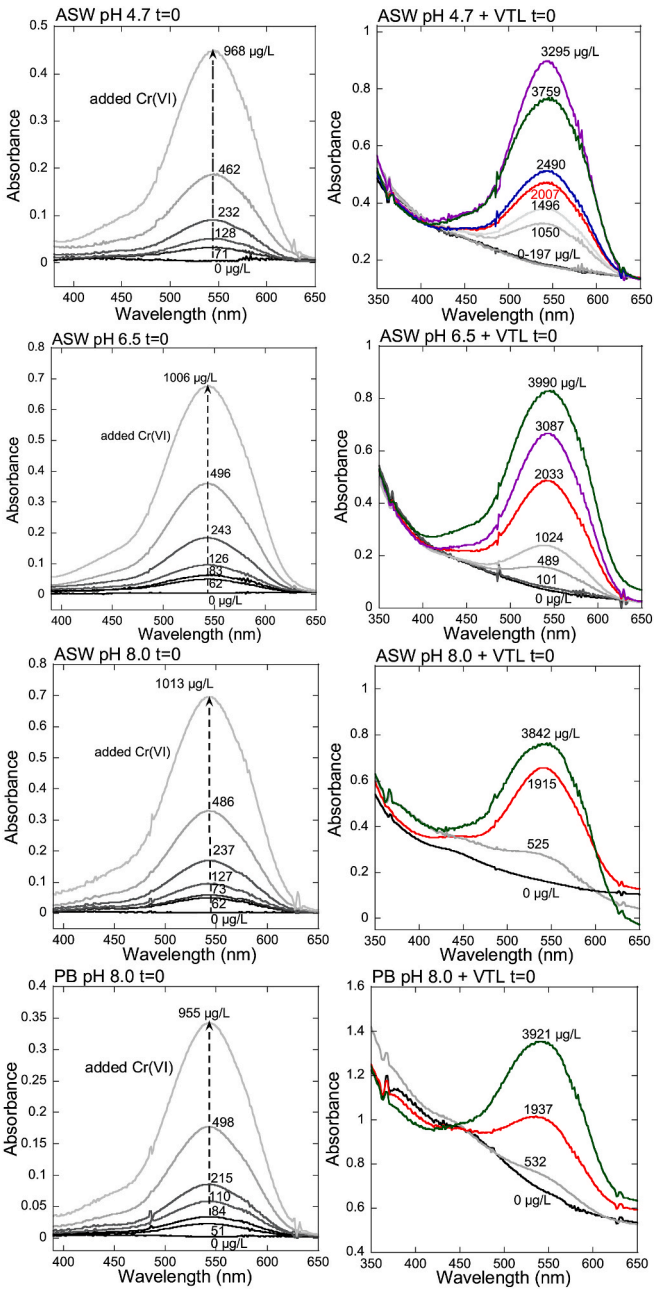

Fig. 2. Representative UV–vis spectra as a function of added Cr(VI) concentrations, immediately after addition of the respective Cr(VI) amount (t = 0), to artificial sweat (ASW) at pH 4.7, 6.5, and 8.0, and deaerated phosphate buffer (PB) at pH 8.0 (left), or these solutions after 24 h incubation with Cr-free vegetable-tanned leather (VTL, right). The baseline has been shifted to overlap in all cases, for clarity.

For the other three solutions, which had been pre-exposed to Cr-free leather, the reduction of Cr(VI) was slower, as Cr(VI) values were measurable after 1 h of reaction time. The pH 6.5 ASW showed the next greatest reduction capacity out of the three in the presence of VTL species, as it displayed the largest reduction at t = 1 compared to its initial t = 0 values. For the two pH 8.0 pre-exposed solutions (ASW and

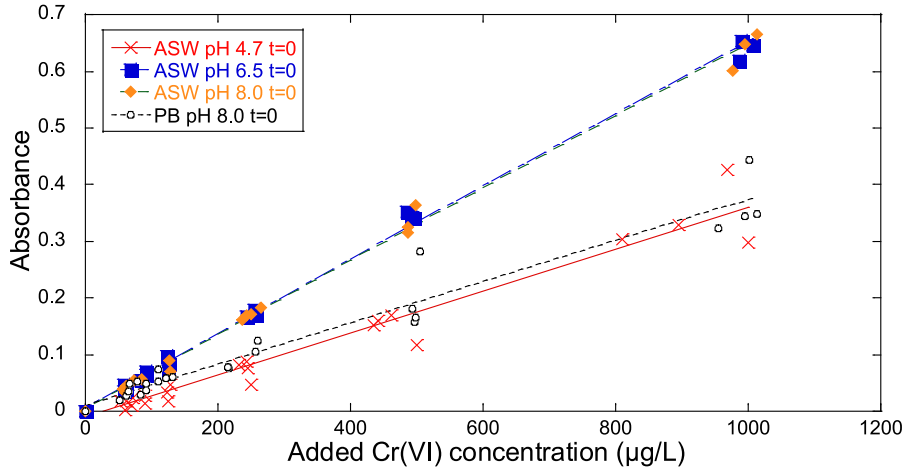

Fig. 3. Three (ASW pH 8.0 and ASW pH 6.5) to four (all other solutions) independent trials of artificial sweat (ASW) at pH 4.7, 6.5, and 8.0, and deaerated phosphate buffer (PB) at pH 8.0 as a function of added Cr(VI) concentration, measured immediately (t = 0) after addition. The lines represent the linear fit of these 3–4 independent trials and represent the calibration curve used for the reduction capacity trials (Fig. 4).

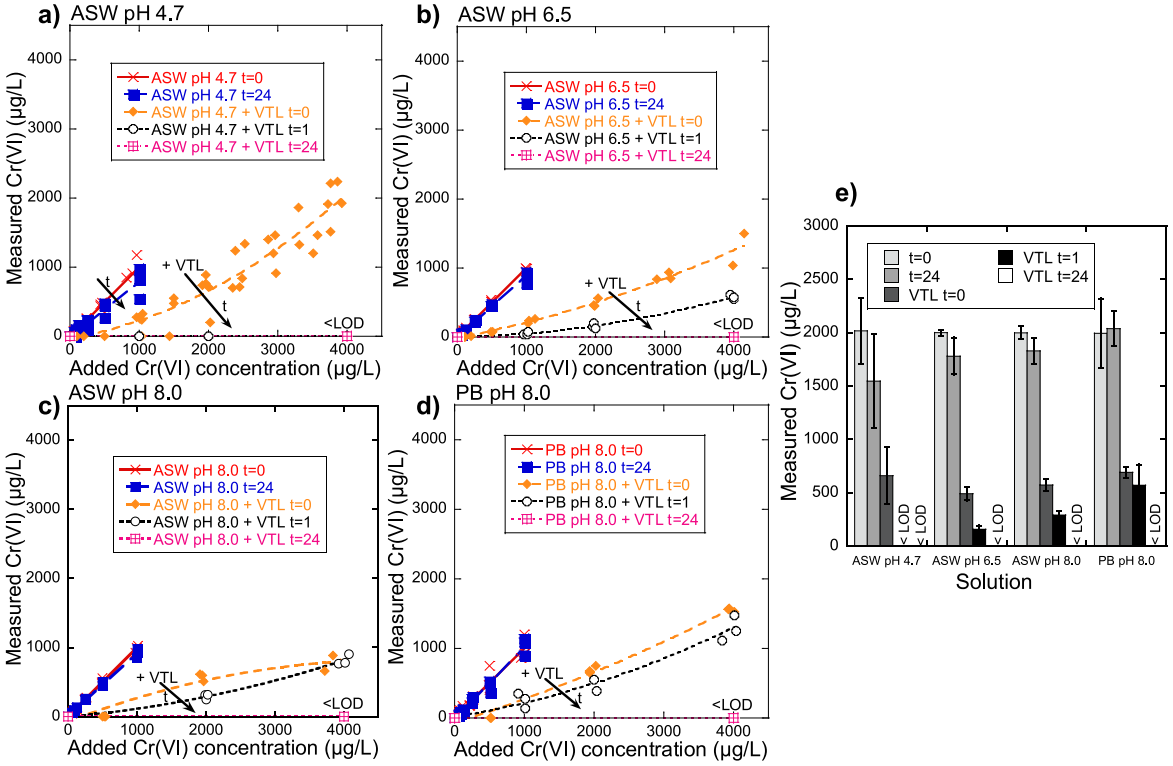

Fig. 4. Measured (recovered) Cr(VI) concentration as a function of added Cr(VI) concentration for artificial sweat (ASW) at pH 4.7 (a), 6.5 (b), 8.0 (c), and deaerated phosphate buffer (PB) at pH 8.0 (d), measured directly after addition of Cr(VI) (t = 0) and after 24 h (t = 24). In addition, the four solutions were incubated with Cr-free vegetable-tanned leather (VTL) for 24 h, after which Cr(VI) was added. These solutions were measured immediately (+VTL t = 0), after 1 h (+VTL t = 1), and after 24 h (+VTL t = 24). All concentrations after 24 h in the VTL-exposed solutions, and after 1 h in the VTL-exposed ASW pH 4.7, were below the limit of detection (<LOD). The different points represent independent trials. (e) Comparative representation of remaining Cr(VI) of a 2000 µg/L added Cr(VI) solution (artificial sweat, ASW, at pH 4.7, pH 6.5, and pH 8.0, and deaerated phosphate buffer, PB, at pH 8.0) after immediate measurement (t = 0), 24 h (t = 24), and for solutions first exposed to a Cr-free vegetable-tanned leather (VTL), measured immediately (VTL t = 0), after 1 h (VTL t = 1), and after 24 h (VTL t = 24). <LOD – below limit of detection. The error bars represent the standard deviation of 3–5 independent trials. For the solutions without exposure to VTL, the concentrations have been linearly extrapolated to 2000 µg/L from 1000 µg/L.

PB), measured Cr(VI) values after 0 and 1 h are similar; however, the PB shows the least reduction. After 24 h incubation for all pre-exposed solutions, a complete reduction of Cr(VI) was observed. For these three solutions, when they had not been pre-exposed to Cr-free leather, a reaction time of 24 h only slightly decreased the Cr(VI) concentration for the solutions at pH 6.5 or higher (pH 6.5 ASW: from a slope of 1.0 to 0.89; pH 8.0 ASW: 1.0 to 0.91), with no change observed for the pH 8.0 PB solution.

4. Discussion

Our results suggest that both the pH and composition of the extraction solution, as well as the co-release of vegetable tannins and other leather species, strongly influence the Cr(VI) reduction capacity of the solution.

4.1. Quantitative comparison

Fig. 4e shows a quantitative comparison of the incubated 2000 μg/L Cr(VI) standard. Although the trials for the solutions without pre-exposure to leather did not include the 2000 μg/L Cr(VI) standard, this value was determined by extrapolating the absorbance value of the corresponding added 1000 μg/L Cr(VI) using the linear t = 0 or t = 24 fit equations of each individual measurement, prior to its conversion from absorbance to Cr(VI) as for all other measurements. Fig. 4e shows that there is no statistically significant reduction of Cr(VI) with time for all four solutions in the absence of leather, but significant time and pH-dependent reduction of Cr(VI) after pre-exposure to Cr-free, non-dyed and non-finished vegetable (mimosa) tanned leather. Without pre-exposure to leather, the pH 4.7 ASW shows the largest reduction capacity (about 23% after 24 h), although still not statistically significant, and the PB shows the smallest reduction capacity. The pH 6.5 ASW shows a statistically non-significant decrease of about 11% after 24 h. The results are in line with Stefaniak et al. (2014), where an artificial sweat solution at pH 5.3 at 36 °C reduced initial Cr(VI) concentrations by about 10% after about 30 h.

The negligible reduction capacity of the PB is expected, as this extraction solution has been optimized for Cr(VI) extraction from leather (Eurofins BLC, 2017; ISO, 2017). This low reduction capacity has also been reported in previous literature (Hedberg et al., 2014). The ASWs may also contain species that contribute to Cr(VI) reduction, such as their differing amino acid components; however, this was not further investigated. To investigate whether the buffering capacity influenced the reduction capacity of the respective solution, the pH after incubation was measured. For the PB, pH 4.7 ASW, pH 6.5 ASW, and pH 8.0 ASW, the average pH values of the solutions without exposure to VTL after 24 h incubation are 8.03 (Blank: 8.04), 4.67 (Blank: 4.71), 5.76 (Blank: 5.79), 7.98 (Blank: 8.00), respectively. For the PB, pH 4.7 ASW, pH 6.5 ASW, and pH 8.0 ASW, the average pH values of the solutions with 24 h exposure to VTL and 24 h incubation with Cr(VI) are 7.53 (Blank: 8.06), 4.65 (Blank: 4.76), 4.46 (Blank: 4.01), 6.79 (Blank: 8.06), respectively. It can be concluded from these values that, *i*) the pH 6.5 ASW does not have a high buffering capacity, that *ii*) exposure to VTL had a varying effect on the solution pH, i.e. some acidifying species were released, and that *iii*) only the pH 8.0 PB has a sufficiently high buffer capacity to keep the solution pH above 7.5 (which is a requirement of ISO 17075) in the presence of VTL.

The added Cr(VI) was most stable in the pH 8.0 PB, which was designed to keep Cr in the hexavalent form. The deaerated pH 8.0 PB supposedly minimizes the oxidation potential of the buffer, i.e. no new Cr(VI) should form during analysis, and no Cr(VI) should be reduced to Cr(III), allowing for an accurate extraction of Cr(VI) (Eurofins BLC, 2017; ISO, 2017; Hedberg et al., 2015). While some consider pH 8.0 PB the ideal extraction solution (e.g. Hedberg, 2020), many studies have been conducted that argue against the use of pH 8.0 PB for Cr(VI) extraction. These studies claim that an alkaline extraction pH can produce false positive results; for example, some studies state that chromate (Cr(VI)) can form during alkaline analysis via oxidation (Ballardin and Iannone, 2013; Font et al., 1999; Long et al., 2000). The alkaline pH can also cause dye bleeding, which can further lead to overestimations of Cr (VI) content (Graf, 2001). It is also noted that pH 8.0 PB may be less relevant for simulating real-life skin exposure as human sweat is naturally acidic (Ballardin and Iannone, 2013). Although there is much controversy regarding the use of PB in the ISO 17075 standard, our work shows that the PB (with the lowest reduction capacity) is the least likely to provide an underestimation of Cr(VI) content on or in test items to be extracted.

Vegetable tanning agents (VTA) have phenolic character that can facilitate antioxidant activity (Ozkan et al., 2015; Palop et al., 2010). When a leather is partially tanned with VTA, Cr(VI) formation is inhibited as the VTA scavenge for free radicals and eliminate the opportunity for Cr(III) oxidation (Palop et al., 2010). Our results displaying the reducing ability of vegetable extracts are consistent with many studies, including those written by Font et al. (1999); Graf (2001); Hauber (2000); Hedberg et al. (2014); Ozkan et al. (2015); Palop et al. (2010); Blázquez et al. (2002). However, although the VTL released effective reducing agents, we observed that Cr(VI) can still be stable for at least 1 h in solutions above pH 4.7 that were pre-exposed to the Cr-free leather. It is also worth noting that other leather species can be responsible for Cr(III) oxidation, i.e. unsaturated fatty acids reacting with oxygen to form peroxides, which enable the formation of Cr(VI), particularly at alkaline pH (Graf, 2001; Hauber, 2000; Long et al., 2000; Pastore et al., 2004).

4.2. Implications for dermal exposure

It is widely known that Cr(VI) has relatively high skin penetration rates, diffusing through the skin entirely after 96 h in simulated sweat (Van Lierde et al., 2005a). In comparison, Cr(III) has a strong affinity for skin and thus has low skin penetration ability. Therefore, as Cr(VI) reduces to Cr(III), the bioavailability of Cr decreases. This process is time-dependent, as is the diffusion of Cr(VI) through the skin. Thus, there is a risk that an extraction procedure that underestimates the initial amount of Cr(VI) formed on surfaces of leather items is underestimating the bioavailable Cr(VI). A real skin exposure scenario is thin film chemistry involving different diffusion and reduction processes with different rates simultaneously. Based on our study, the pH 8.0 PB would be least likely to underrepresent the amount of Cr able to penetrate through the skin, as it does not change the chemical speciation of Cr on its own. All solutions, because of the relatively long (hours) extraction times employed in different standardized tests, are at risk to underestimate Cr(VI) in or on leather in the presence of co-extracted typical leather species. Most tanning procedures involve both Cr tanning and vegetable or synthetic tannins with reducing properties. The investigated ASWs, with pH 4.7 having the greatest reduction capacity, may change the amount of initially bioavailable Cr(VI); however, it is not clear whether this reduction is representative of what would occur during dermal exposure.

4.3. Limitations of this study and future studies

The assumption was made that at t = 0 (for the standard calibration curves), no reduction occurred to form Cr(III); all Cr present in solution is in the form of Cr(VI). This assumption was made for analytical purposes and is not entirely valid as some reduction does occur at t = 0 (Fig. 3) in the solutions without co-released leather species, however, it is very minimal. This negligible t = 0 reduction in slope, cannot be quantified based on the highest observed slope, because the slopes of the calibration curves at t = 0 are also influenced by other factors, such as the solution transparency and temperature. All subsequent time points and the solutions after exposure to VTL were compared to that t = 0 time point, which was based on the averaged calibration curve of all data points obtained from 3 to 4 replicate experiments, accounting for uncertainty among various measurement days. This inter-measurement uncertainty was greater for two solutions (PB pH 8 and ASW pH 4.7), with a possible error fortification of about 20%; however it did not affect the main conclusions.

Another limitation of the study was a red particulate matter that formed upon the addition of DPC and phosphoric acid to the pH 8.0 solutions after contact with the VTL. This increased the turbidity of the samples, which could have affected the spectroscopic data, required

alterations to the spectrophotometric procedure (see section 2.6), and reduced consistency between experiments of varying pH values.

Future studies should aim to investigate other ASWs to provide a reference for researchers to consult regarding suitability for their specific experimental needs. For example, it has been shown that methionine is the most important component in artificial sweat to reduce Cr(VI) to Cr(III) (Van Lierde et al., 2005b), however, none of the here investigated ASWs contained methionine.

Experiments should also be designed with thin film chemistry in mind or direct solid-state Cr surface analysis, instead of relying on bulk solution extractions only.

5. Conclusion

The aim of this work was to investigate the ability of different extraction solutions, including different ASWs (with pH values ranging from 4.7 to 8.0), and a standardized extraction solution, to reduce Cr(VI) as a function of time and in the presence of typical species released from leather items. The following main conclusions were drawn:

1. None of the four extraction solutions reduced Cr(VI) significantly up to 24 h, but the greatest reduction (about 23%) was seen for the most acidic solution, ASW pH 4.7.
2. When pre-exposed to a Cr-free vegetable (mimosa) tanned leather, which was not finished or dyed, all solutions reduced Cr(VI). Reduction increased with time and decreasing pH.
3. Deaerated phosphate buffer at pH 8.0 best preserved the initially added Cr(VI).
4. After 24 h of reaction time, all solutions that had been pre-exposed to the Cr-free vegetable tanned leather, reduced 4000 μg/L Cr(VI) completely. However, after 1 h of reaction time, Cr(VI) was still detected in all solutions equal or above pH 6.5.
5. A combined effect of buffering capacity (and pH value) of the extraction solutions with co-released leather species is evident in a time-dependent reduction of Cr(VI). This data provides information of relevance to design and interpretation of extraction tests aimed to simulate short-term skin exposures of rapidly skin-permeating Cr (VI).

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CRediT authorship contribution statement

Alexandra Wright: Investigation, Formal analysis, Writing – original draft. **Lila Laundry-Mottiar:** Writing – review & editing, Supervision. **Yolanda S. Hedberg:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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