A Study on the Reliability of UNI EN ISO 17075 Method for the Determination of the Cr(VI) Content in Leather

by

Alberto Ballardin and Laura Monica Iannone*

Research consultant for I.T.I.S. G. Galilei and member of AICC

Arzignano, Italy

*I.T.I.S. G. Galilei and member of AICC**

Arzignano, Italy

Abstract

The UNI EN ISO 17075 method for Cr(VI) detection in leather presents several drawbacks. One of these is the choice of an alkaline extraction pH, which produces severe false positive results.

Summary: The hexavalent chromium content in 14 leather samples, resulted positive to the presence of Cr(VI) according to the UNI EN ISO 17075 method in two certified laboratories, was re-determined, using the official extracting method at pH 8 and a different phosphate extraction buffer at pH 4.4 containing 5% NaCl. The well-known transient nature of Cr^6, that is the decrease of its amount in leather during time, required a re-activation by thermal treatment before analysis. The results show that the official method systematically gives false-positive values and that 10 of the 14 examined leather samples, when extracted with a buffer at pH 4.4, proved to have acceptable levels of Cr(VI). In addition we found that Solid Phase Extraction (SPE) cartridges, either normal or end-capped, absorb about 10% of chromate and this indicates that the calibration curves should be obtained after filtering each standard with the SPE employed.

Introduction

The possible presence of hexavalent chromium in chrome tanned leathers was discussed in 1995.[1] This finding raised concern among tanners owing to the toxicity and possible mutagenic and carcinogenic effects of hexavalent chromium. The UNI EN ISO 17075 method is the last evolution of the former DIN 53314 and IUC 18 methods in which the main modifications were the use of 47 mm nylon filters, extraction through SPE cartridges and the increase of optical path of the cells. The acceptability level of 3 mg/Kg for commercial leathers remained unchanged in all three methods. This limit is a trace level and the spectrophotometric detection was prone to severe optical limitations.

In a short time many papers[2-5] confirmed the presumption of unreliability of the DIN 53314 and IUC 18 methods, mainly due to interferences coming from turbidity or the presence of colored substances extracted from leather, that led to an over-estimation of Cr(VI). In 1999 J. Font[2] added another drawback, suggesting that about 60% of the detected chromate was formed during analysis, giving false-positive results. The same objection was made by Long et al.[3]

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*Corresponding author e-mail: lauram_iannone@yahoo.it

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In 2000 Ballardin and Xompero [6] proposed a reaction for the formation of Cr(VI) inside the leather:

1) $\mathbf{3\ ROOH + 2\ Cr(OH)_3 + 4\ OH^- = 3\ ROH + 2\ CrO_4^{2-} + 5\ H_2O}$

where ROOH is a peroxide formed by the reaction of unsaturated fat-liquoring oils and oxygen.

This equation was proposed for the alkaline environment of the extraction method. Owing to the acidic environment in chrome-tanned leather the reaction should be rewritten as:

2) $\mathbf{3\ ROOH + 2\ Cr^{3+} + 8\ OH^- = 2\ HCrO_4^- + 3\ H_2O + 3\ ROH}$

These equations clearly point out the fundamental influence of the pH in the oxidation of Cr(III) to Cr(VI).

In a subsequent study[7] it was confirmed that the reaction between Cr^{3+} and a synthetic peroxide strongly depends on the pH of the medium with an almost exponential increase of Cr(VI) formation at pH close to 8. Moreover the plot of Cr(VI) concentration versus pH had the same shape of that obtained on leather, supporting the proposal of the role of peroxides in chromium oxidation. Pastore et al[8] proposed an analytical method of Cr(VI) detection that employed IC (Ionic Chromatography) which drastically increased the sensitivity and specificity of Cr(VI) detection. They used an acidic extraction buffer, with a pH very close to the pH of normal aqueous leather extract and human sweat. In the same publication the false-positive results of the official method were clearly evidenced.

In the present work we conducted an analysis of the hexavalent chromium content in commercial leathers by comparing acidic and basic extraction results. The aim of the research was to verify if our previous conclusions, based on a model system, could be confirmed in commercial leathers. Since this paper is dedicated to the influence of the extraction buffer pH on Cr(VI) detection we employed the classical spectrophotometric technique of the official method, now free from optical interferences by the introduction of filtration over 47 mm filters to eliminate turbidity and the elimination of organic colored substances with the filtration on Solid Phase Extraction cartridges.

MATERIALS AND METHODS

Reagents and Instruments

1. Diluted phosphoric acid (H_3PO_4) solution: 700 mL of 85% phosphoric acid diluted to 1 L with water;

2. 2% 1,5-diphenyl carbazide (DFC) solution: 2 g of DFC was introduced into a 100 mL flask , brought to the mark with acetone and a drop of glacial acetic acid was added;

3. Bi-distilled water: the water for the preparation of all solutions was obtained by a double distillation of de-ionized water, followed by a 5 minutes bubbling of N_2 with a flux of 50 (+/-10) mL/min;

4. Buffer at pH 8: 22.8 g (0.1 mole) of $K_2HPO_4{\cdot}3H_2O$ were dissolved in 750 mL of H_2O, the pH was adjusted to 7.5-8.0 with diluted H_3PO_4, dissolved air was eliminated by bubbling N_2 for 5 min at a flux of 50 ml (+/- 10 ml)/ min and the volume was brought to 1 L with water;

5. Buffer at pH 4.4 : 0,1 mole (11.997 g) of NaH_2PO_4, and 50 g of NaCl were dissolved in 750 mL of H_2O, the pH was brought to 4.4 with diluted H_3PO_4 , the solution was degassed as in point 4 and the volume adjusted to 1 L;

6. 0.45 mm nylon filters (Φ 47 mm)

7. Cartridges C18 SPE Agilent Accubond[II] or normal C18 Agilent;

8. Spectrophotometer UV/VIS: Perkin Elmer Lambda 25 with 2 cm optical path glass cells.

9. Oven.

10. Waters Vacuum filtration system.

Analysis Method

1. The leather samples are grinded to powder and heated at 80°C in an oven, without humidity control, for 24 hr;

2. 100 mL of buffer (pH 7.5-8.0) for alkaline extraction are poured in a bottle and bubbled with N_2 for 5 minutes. The same procedure is employed with 100 mL of the buffer at pH 4.4 (+/- 0.2) for the alternative acidic extraction.

3. 2,000 g of leather powder, heated as point 1, and a magnetic bar are inserted, the bottle is tightly capped and the content is stirred for 3h (+/- 5 min);

4. The extract is then filtered through a nylon filter in a glass bottle with cap to eliminate the leather powder. The pH is controlled (7.5-8.0 or 4.4);

5. 20,0 mL of solution are collected and filtered in the vacuum system through SPE in to a 50 mL flask under a mild vacuum. The cartridge is then washed with 10 mL volume of buffer, added in small portions, collecting the washings in the 50 ml flask;

6. The flask is removed from the vacuum system; 1 ml of the phosphoric acid solution and 1 ml of the 2% DFC solution are added. Then the flask is filled to the mark with the buffer;

7. After 15 min (+/- 5 min) the absorbance of the sample is measured at 540 nm against a blank prepared as in point 6, but without addition of DFC. Our cell had an optical path of 2 cm but higher path lengths can be employed to increase sensitivity;

The concentration of Cr(VI) in the sample is obtained from a calibration curve obtained by filtering each standard on the same kind of SPE cartridges, as in point 5.

Samples

The leather samples, which tested positive by the UNI EN ISO 17075 method for Cr^{6+} (i.e. a level superior to 3 ppm), were kindly supplied by two local and certified Laboratories. Since the conditions and storing time of the original samples were unknown, we checked the level of Cr(VI) using the official method and we could confirm the transient nature, viz. the decrease of analyte in time, since all the values were lower than those found in the initial analysis of the samples. In order to bring all the samples to an identical starting condition we submitted the samples to a thermal treatment at 80°C for 24 hours (a method proposed in Europe with the CEN/TC 309 procedure to determine the tendency of a leather to form hexavalent chromium).

RESULTS AND DISCUSSION

Comparison of Cr(VI) Content in Leathers Extracted at pH 8 and 4.4

It is clear that all the samples extracted at pH 8.0, except sample 4, contained amounts of Cr(VI) beyond the limits fixed by current regulations. Sample 4 showed, instead, a total and irreversible reduction of Cr(VI) to Cr(III) in time. The acidic extraction strongly reduced the amount of hexavalent chromium detected and in 9 cases the leather samples could be judged acceptable for marketing. We have already shown,[8] by employing leather spiked with known amount of Cr(VI), that Cr(VI) is formed during analysis in alkaline conditions and that the oxidized form is stable in mild acidic pH.

The aim of this paper (viz. to confirm that Cr(VI) was produced in alkaline conditions even in real leather) is verified by the results shown on Table 1, which also shows that if chromate does exists in leather it is detected even at pH = 4.4, notwithstanding the reducing capability of leather.

The Yield of Filtration with SPE Cartridges

The official method prescribes that filtration on SPE must recover more than 90% of the present Cr^{6+}. In the course of our research we were not able to find a brand of cartridges fulfilling this requirement, even using end-capped material. Table II shows the loss of Cr(VI) obtained with the use of different brands of cartridges.

TABLE I
Comparison of Cr(VI) content in leathers extracted at two different pH's.

	UNI EN ISO 17075	NEW METHOD
	pH 8	pH 4.4
Sample	ppm	ppm
1	**70.2**	**5.7**
2	25.8	1.4
3	**145.9**	**46.6**
4	0.3	0.0
5	30.2	0.6
6	23.7	0.0
7	43.7	0.0
8	**90.3**	**83.9**
9	9.4	0.0
10	6.4	0.8
11	12.0	0.3
12	7.6	0.0
13	4.9	0.1
14	**71.1**	**16.1**

TABLE II
Loss of Cr(VI) as measured by the variation of extinction coefficient of calibration curves.

Cartridge	Δε at pH= 4.4	Δε at pH = 8.0
Normal SPE (type 1)	10.6%	7.3%
Normal SPE (type2)	10.6%	7.3%
End capped SPE	17.4%	8.7%

The loss of Cr(VI) in acidic medium remained in the order of 10.6%, an unacceptable result, while it was 7.3% in alkaline condition, a correct value.

This loss of Cr(VI) during filtration led obviously to an under-estimation of Cr(VI) in leather at a level comparable to the standard deviation of the method (theoretically at least one tenth of the 3 ppm limit to insure measurability). The worse results are observed for end-capped cartridges, thus the use of normal cartridges is recommended. We then decided to perform the filtration on SPE for all the standards used to obtain the calibration curve in order to correct for the loss of analyte during analysis.

DISCUSSION

Our old doubts on the various modifications of the original DIN 53314 and I.U.C. 18 methods were:

1. The choice of an alkaline extraction buffer so different from the acidic character of human sweat (pH around 5) and of chrome tanned leather matrix,

2. The use of a spectrophotometric analytical technique, with low sensitivity and a high tendency to be affected by optical interferences; the choice was based on the simplicity of analytical procedures and the low cost of equipment, but at the cost of reduced sensitivity and specificity proper of other available analytical methods,

3. The official method is based on the supposed stability of the analyte at pH 8. However we showed[8] that the specie is a transient in leather and this dictates the use of analytical techniques for transient (viz. the definition of storing conditions and time interval between collection of samples and analysis to avoid modifications of the original analyte content),

4. The choice of an acceptability limit of 3 ppm doesn't come from a medical/biological investigation of a dose-effect study on living systems but is a mere consequence of the choice of the analytical methodology; moreover we must observe that the sensitivity of the method was improved by increasing the optical path of the cells employed (up to four times), but the limit was not accordingly reduced,

5. The auto-reference of the method that protects itself from disapproval by the affirmation that the hexavalent chromium content in leather is that determined by the method itself.

This seems to be a mere semantic criticism but in this paper we confirmed that the method is wrong and so are the levels of Cr(VI) determined by its use. The Cr(VI) in leather is what it is really present and, hence, it must be determined by a different, sensitive and robust analytical procedure, which is already available.[8]

Point 1, which is decisive in causing the false positive result of the official method, deserves a short explanation. We decided to employ an acidic extraction buffer since it was very similar to the pH of the aqueous extract of tanned leather (3.5<pH<4.0, according to the UNI EN ISO 4045 method). The 4.4 value is also very close to the pH of human sweat. This limit comes from the need to have a positive charged chrome leather to insure fixation of all the many anionic substances (especially dyes and fat liquors) employed in tannery operations. To insure a positive charge the extraction must be conducted at a pH inferior to the isoelectric point of chrome tanned leather (5.5-6.0). As far as we know the alkaline pH extraction was chosen to decrease the redox potential of Cr(VI), which could cause false negative results. But we observed that an alkaline pH does not only loosen the bond of all anionic molecules from leather but also transform Cr^{3+} bound to collagen into $Cr(OH)_3$, so de-tanning the leather. Another important consequence is the increase the oxidability of Cr(III) to Cr(VI).

In this paper's objections, 1, 3 and 5 found experimental support, while point 2 was already resolved in 2004[8] by the employment of the well established ion-chromatographic technique with post-column reaction. The answer to point 4 is a bio-medical issue and we are actively working on this aspect of Cr(VI) toxicity in leather.

Preliminary examination of published safety assessment,[9,10] indicates that the risk of Cr^{6+} exposure greatly depends on it's physical state with the higher danger coming from the exposition to the solid form (essentially powder or aerosol inhalation which have a proven carcinogenic and mutagenic effects on humans). The absorption of aqueous solution of Cr(VI) (both as drinking water and by contact with wet materials, as humid leather) seems to have different and less dangerous consequences.

A possible objection can be raised about our choice of a very drastic 80°C initializing treatment that can truly modify the leather matrix.[11] However leather technicians observed that the present conditions of transportation of leather's material in over-heated metallic containers and long periods of conservation in hot climate are closely approached by our choice. The 80°C pre-treatment (coming from the previously mentioned CEN/TC 309 method for footwear) is nowadays questioned but the decrease of chromate with acidic extraction remains experimentally proved. The corrections to the method that we propose seem adequate to insure the applicability of a correct, simple and economic determination of Cr(VI) level in chrome tanned leathers.

The Ionic Chromatographic method[8] clearly remains far superior in sensitivity and specificity.

CONCLUSIONS

It is clear that the UNI EN ISO 17075 method is affected by a systematic over-estimation of Cr(VI) content in leather and that the safety level established does not have a medical/ biological support. Although we dislike to criticize an UNI EN ISO analytical method, we are convinced that the relevancy of safety, economical and chemical consequences of systematic false-positive results imposes a severe reconsideration of the entire procedure, starting with the essential modification of the extraction buffer pH and composition and the use of a calibration curve obtained in exactly the same filtering conditions as the actual samples.

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REFERENCES

1. Nickolauss, G.; Does leather contain chromium? XXII Congress of IULTCS 47, 1995
2. Font, J., et al.; Presence of chromium(VI) in sheep skins: influence of tannery processes. *J. Soc. Leath. Tech. Chem.* **82**, 91-95, 1998
3. Long, J., Cory, N. J., Wood, C. B.; Potential chemical mechanisms causing false positive results in hexavalent chromium determination. *J. Soc. Leath. Tech. Chem.* **84**, 78 -74, 2000
4. Jambunathan, S., Dasgupta, P. K.; Determination of hexavalent chromium by the diphenyl-carbazide (IUC 18) procedure: pitfalls and refinements. *J. Soc. Leath. Tech. Chem.* **84**, 63-73, 2000
5. Milacic,R., Scancar, R. J., and Urbanc, M.; Is spectrophotometry a reliable technique for determination of Cr(VI) in leather samples? *J. Soc. Leath. Tech. Chem.* **82**, 91-94, 1998
6. Ballardin, Alberto, lberto, X. M.; Studio sulla formazione del cromo esavalente nelle pelli. *Cuoio, Pelli, Materie Concianti* ***2*****,** 156-165, 2000
7. Concato, D.; Diploma di tesi available at www. istitutoconciario.com
8. Pastore, P., Ballardin, A., Favaro, G., and Danieletto,D.; Evidence of Cr(VI) formation during analysis of leather. Proposal of an alternative method of analysis through the ion-chromatographic method approach and post-column reaction. *Talanta* **63**, 941-947, 2004
9. Agency for toxic substances and disease Registry (ATSDR); Toxicological profile for Chromium. U.S. Public Health service, Department of Health and Human Services, Atlanta, GA, 1993
10. U.S. Environmental Protection Agency; Integrated Risk information system (IRIS) on Chromium. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, Cincinnati, OH. 1993
11. Girardi, V., Galvanese, G., Naviglio,B., ZolloF.; Studio sulla possibilità di formazione del Cr VI dopo invecchiamento del cuoio. *Cuoio Pelli Materie Concianti* **5**, 297-305, 2009