

Mechanisms for false hexavalent chromium determinations in aqueous extracts from leather and soil

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Before the common use of Atomic Absorption Photospectrometry and Inductively Couple Photospectrometry, being instrumental methods of analysis, chromium was determined by titration in an oxidation/reduction reaction (ALCA Method D 10) or oxidation of chromium to hexavalent state and determination using diphenylcarbazide¹ as a coloured indicator.

The titration method is best suited to large amounts of chromium, such as found in chrome tanned leather. However, the diphenylcarbazide method is much better at small quantity determinations, and has been widely tested and used by many researchers², specifically as a reliable indicator for hexavalent chromium.

Nevertheless, considerable discussion and research has taken place into the potential weaknesses and accuracy of the diphenylcarbazide determination of hexavalent chromium in leather^{3, 4, 5}. Despite these reservations, the general attitude has been to accept that hexavalent chromium - as determined by diphenylcarbazide methods - is real and hazardous⁶.

Diphenylcarbazide as an indicator

The use of diphenylcarbazide has evolved over many decades. Originally, the methods for hexavalent chromium were largely based on ashed materials, therefore organic substances were of no consequence. But recently the use of the method on extracts from leather, soil and other heavily organic substrates has become common place.

References in the old methods to interference from iron, vanadium and other metals have been interpreted to mean that those were the most logical and reasonable agents of concern for false positive results. In fact, those were the only major interfering substances in ashed or mineral samples.

However, long before it was used for hexavalent chromium analyses, diphenylcarbazide had been used as a coloured indicator for organic compounds. Alkaloids are plant metabolic products, and alkaloids such as emetine and aldehydes are listed in the Merck Index as common substances detected with diphenylcarbazide⁷.

Discrepancies between hexavalent and total chromium values in samples

Perhaps the first and most critical determination of hexavalent chromium in an extract from an organic matrix was the soil analysis by Bartlett and James back in the late 1970s⁹. This used diphenylcarbazide as an indicator. Believing that the coloured

complex produced in these investigations was proof that hexavalent chromium could form and exist in typical soils, the authors started an investigation that has continued with other researchers to this day.

One peculiar feature of this work was the strongly noted point that the positive hexavalent chromium value from the soil samples disappeared, or was destroyed, by drying the soil before analysis. The analysis, therefore, had to be performed on fresh, moist soil samples, being typically rich in organic compounds with slightly acid pH values.

But these conditions do not fit with the general understanding of hexavalent chromium chemistry. Indeed, most research clearly documents that such conditions would rapidly and definitely reduce any hexavalent chromium to the trivalent state^{10, 11}.

Notwithstanding, although no significant validation of the actual chromium content of the extract was ever demonstrated, the concern for environmental risk sustained a need for caution and regulation.

Degraded organics: source of false hexavalent chromium determinations?

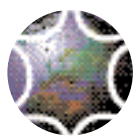
Of course, the nagging question always persisted as to why this coloured response disappeared when the soil was dried prior to analysis. Could the fugitive agent have been volatile?

In addition, personal experiments with fresh soil confirmed that diphenylcarbazide did indeed produce a bright pink colour with many soil extracts. Moreover, in these examples, chromium (VI) concentrations were often indicated at greater levels than the total chromium content of the solution as determined by atomic absorption spectrophotometry.

Decades of tanning research into chromium chemistry¹²⁻¹⁵ have been based on consistent oxidation/reduction principles that appear to be violated by an existence of hexavalent chromium in wet, acidic soils. Further, logic suggests that heating the sample is the only point at which hexavalent chromium could possibly form¹⁶ as opposed to a simple drying procedure.

Then there is the issue of all the organic reductants and complexing agents commonly present in soil. For instance, tannins are very common as by-products of plant growth in soils, being strong reductants for hexavalent chromium, and complexing agents for trivalent chromium and most other metals¹⁶⁻¹⁸.

Green plants¹⁹, bacteria²⁰, and soil fungi²¹ also produce a huge range of complex organic compounds as natural metabolic products. Although the complete array of these compounds is



almost infinite and variable with specific organisms, the organic acids that are typically reflected in fats and oils^{22, 23} are common in some form to virtually every organism. These compounds would be present in the soils from diffusion through membranes and tissues, and even decaying organisms.

And regarding aged leathers, these contain degradation products from proteins, retanning agents and other organic substances as a result of oxidation and photolysis. In particular, fats are present and subject to degradation, and metals such as chromium often contribute a catalytic effect to this deterioration⁸.

Clearly, extracts from complex organic matrices, including both soils and leather, may contain a significant variety of soluble, complex organic chemicals. Moreover, amines and organic acids are common to diverse living organisms, deteriorating fatty materials and proteins, and complex processing chemicals.

Since there is a significant history of such organic compounds being active with diphenylcarbazide colour development, an investigation into potential false positive hexavalent chromium determinations commenced.

Observations from routine hexavalent chromium work in the laboratory

The following details were noted concerning diphenylcarbazide indicator and its use under practical laboratory conditions:

- Most procedures for preparing the diphenylcarbazide indicator specifically require that the indicator solution be prepared fresh, at least weekly. Most analysts resist this requirement since they seldom use the indicator that quickly. The tendency is to put the indicator in amber or brown glass containers and refrigerate it to keep it fresh. Even months after it has been prepared, analysts are quick to point out that it is still effective and produces good colour with hexavalent chromium standard solutions. What the analyst is eager to ignore is that the clear solution is ever so slightly yellowing and that it is now much more sensitive than when fresh to compounds other than hexavalent chromium.
- One sample of prepared diphenylcarbazide indicator was kept in the refrigerator for more than six months. The solution was stored in a 50 ml volumetric flask which was a dark amber colour. By sheer accident the flask was dropped on a freshly cleaned and waxed concrete floor. A deep, dark purple stain developed immediately and remained for over a decade, as bright and purple as the day it formed.

Of course, after cleaning with typical detergents the floor was most likely alkaline - and diphenylcarbazide is much more reactive to organic compounds under alkaline conditions. Although there is no absolute proof, there is little likelihood of any hexavalent chromium on that floor, and this did not appear to be a chromium reaction.

- Another volumetric flask of diphenylcarbazide solution aged well over six months and kept refrigerated during that time, was poured directly onto a sheet of white typing paper. The paper turned brilliant pink. The paper had not been exposed to any hexavalent chromium in the laboratory.
- During the preparation of blanks for hexavalent chromium

analysis, a short but distinct flash of pink or purple colour occurs when the indicator is first added to the distilled water acidified with acetic acid. This colour does not persist, being a brief burst that is easily missed.

- With these peculiar observations, and the subtle inferences that diphenylcarbazide had once been more commonly used with organic compound detection, a deeper research was warranted.

Producing colorations with diphenylcarbazide indicator, in the absence of hexavalent chromium

Since hexavalent chromium determination remained the focus, the conditions of colour development were kept similar to those used in the chromium procedure, that is, strongly acidic solutions. Using the resources of a modestly stocked chemical supply room, reagent grade chemicals were selected for testing for colour development with diphenylcarbazide solution as prepared for hexavalent chromium analysis. The following key matters were observed:

THE INFLUENCE OF ORGANIC ACIDS ON COLORATION

When the diphenylcarbazide solution was added to the water and acetic acid blank, an instantaneous flash of pink colour was produced (as described). However, propionic and butyric acids produced a brighter and longer lasting pink colour. Starting with short chain organic acids and progressing to medium chain length acids, the diphenylcarbazide produced stronger and more permanent colour.

Simple organic acids with 6 to 8 carbons appeared to give strong, stable pink colour on the addition of diphenylcarbazide solution. The limited water solubility of the higher molecular weight organic acids made any continuation futile since these acids form an oily layer or phase separation with water.

These test acids were simple, straight chain, single carboxyl group acids. The fact that they reacted with diphenylcarbazide under the same sample conditions as hexavalent chromium indicates:

- a direct potential for creating false chromium analysis.
- the suspicion that a great many other simple plant and microbial acids might also be capable of giving a coloured reaction with this indicator.

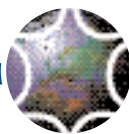
THE INFLUENCE OF AMINES ACIDS ON COLORATION

Simple amines also produced a strong, permanent colour when added to diphenylcarbazide indicator solution. The colour was more red than pink, but at low concentrations might be difficult to distinguish.

Although less systematically evaluated, methyl and ethyl amines were notably good colour producers with the indicator solution. Other common analytical amines did not produce colour with the indicator under these conditions. Longer chain length compounds of the simple amines were not readily available, and therefore were not tested.

False positive hexavalent chromium results from leather and soil extracts

Clearly organic acids and amines do react with diphenylcarbazide to produce a colour that could be wrongly interpreted as hexavalent chromium under the usual analytical conditions. Although these compounds may not have been a concern in ashed samples, the



modern practice of extracting organic matrices - such as soil and leather - could certainly lead to the introduction of these organic compounds into analytical solutions.

In those early experiments back in 1979 by Bartlett and James, the acids from plants and microbes may have been extracted in soil extracts. Volatile organic acids that evaporated when the samples were heated during drying could easily account for the unexpected disappearance of the positive diphenylcarbazide result.

In the current dilemma of hexavalent chromium in leather extracts, the condition is strongly linked to the oxidation of fats in the leather matrix, one of the primary products of fat deterioration being short chain fatty acids. Products of this type have been shown to create colorations with diphenylcarbazide indicator, similar to those produced in the determination of hexavalent chromium.

The use of diphenylcarbazide as an indicator for hexavalent chromium in extracts, where a high level of precision is needed, is therefore thrown into serious doubt. Furthermore, the detection of low levels of hexavalent chrome in extracts from either soil or leather samples may be attributed to false positive results. ●

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