

False-positive result when a diphenylcarbazine spot test is used on trivalent chromium-passivated zinc surfaces

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Summary

Background. A colorimetric 1,5-diphenylcarbazine (DPC)-based spot test can be used to identify hexavalent chromium on various metallic and leather surfaces. DPC testing on trivalent chromium-passivated zinc surfaces has unexpectedly given positive results in some cases, apparently indicating the presence of hexavalent chromium; however, the presence of hexavalent chromium has never been confirmed with more sensitive and accurate test methods.

Objectives. To examine the presence of hexavalent chromium on trivalent chromium-passivated zinc surfaces with a DPC-based spot test.

Methods. A colorimetric DPC spot test was used for the initial detection of hexavalent chromium on new and 1-year-aged trivalent chromium-passivated zinc surfaces. Then, X-ray photoelectron spectroscopy (XPS) was performed for all samples.

Results. The DPC spot test indicated the presence of hexavalent chromium in aged, but not new, trivalent chromium passivation on zinc; however, subsequent analysis by XPS could not confirm the presence of chromium in a hexavalent state.

Conclusions. Unintended oxidation of DPC induced by atmospheric corrosion is suggested as a possible reason for the false-positive reaction of the DPC test on a trivalent chromium-passivated zinc surface. Further validation of the use of the DPC test for chromium-containing metallic surfaces is required.

Key words: contact allergy; DPC spot test; EU regulation; false-positive result; hexavalent chromium; trivalent chromium passivation of zinc.

Chromium allergy and dermatitis are relatively frequent among patch test populations, and have been observed in approximately 4% of patients within the European Surveillance System on Contact Allergies network, and in 5% of patients from the North American Contact Dermatitis Group (1, 2). Historically, the most important source of chromium exposure has been cement, but, following regulatory intervention first in Denmark and later in the

whole EU, leather and various metal alloys, in particular those that are chromium-plated, are becoming relatively more important (3). Although both trivalent and hexavalent chromium may result in allergic contact dermatitis (4, 5), the hexavalent form is by far the most common contact sensitizer. This is most likely explained by the relative inability of trivalent chromium to be absorbed through intact skin, which hexavalent chromium easily penetrates. However, when the skin barrier function is compromised, trivalent chromium can also penetrate to the viable layers of the epidermis and cause dermatitis (6).

Recently, a screening spot test based on diphenylcarbazine (DPC) was developed to detect any release of hexavalent chromium ions from diverse surfaces (7–9). The DPC test is intended to help clinicians rapidly

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identify items that release hexavalent chromium, and may therefore be causing, or contributing to, dermatitis. Although there has been little experience with its use, a validation study showed that the test was very sensitive and produced no false-positive test reactions (9). Surprisingly, some reports of its use have suggested the presence of hexavalent chromium in the trivalent chromium-passivated surfaces (10, 11), although a strong oxidizer, high pH and/or a significant temperature increase is normally required to bring chromium to its highest state of oxidation, which was not the case in the reports. First, Rochester and Kennedy (10) reported hexavalent chromium in the condensate from trivalent chromium-passivated articles after accelerated corrosion tests in a salt fog chamber (Neutral Salt Spray Test). Li et al. (11) also reported that the presence of hexavalent chromium developed over time in objects treated with several methods of trivalent chromium passivation after using the Neutral Salt Spray Test, and even after several days of storage under ambient conditions. Moreover, different types of trivalent chromium passivation seemed to result in different concentrations of hexavalent chromium in the coating. In both cases, DPC was used for identification.

The above observations call into question either the validity of DPC testing, or the safety of trivalent chromium passivation, as hexavalent chromium may cause allergic contact dermatitis and has carcinogenic potential. In the present study, we examined zinc-plated samples with hexavalent and trivalent chromium passivation by using the DPC spot test and X-ray photoelectron spectroscopy (XPS), respectively. This was performed to examine whether hexavalent chromium does appear on trivalent chromium passivation, or whether DPC testing could yield false-positive results.

Materials and Methods

Zinc-plated samples for passivation were obtained by electroplating of carbon steel (St37) panels. Prior to plating, substrates were electrochemically degreased [anodic degreasing in a solution containing 59 g/l sodium hydroxide (CAS no. 1310-73-2) and 14 g/l sodium metasilicate (CAS no. 6834-92-0)], and activated in a solution of dry acid [sodium hydrogen difluoride (CAS no. 1333-83-1) and sodium hydrogen sulfate (CAS no. 10034-88-5)]. The samples were plated in a commercial weak acidic electrolyte (SLOTANIT OT-1; Schlötter Galvanotechnik Geislingen/Steige, Germany) bath for 10 min at 3 A/dm², resulting in a zinc coating thickness of 6–8 µm. After plating, the samples were brightened with a 1 vol% solution of nitric acid (CAS no. 7697-37-2) and subjected

to passivation. For the trivalent chromium passivation, a commercial trivalent chromium passivating solution (TRIPASS PK-3; MacDermid Scandinavia Aaby, Sweden) was applied, with an expected passive film thickness of 30–40 nm (12). For the hexavalent chromium passivation, a chloride-accelerated hexavalent chromate solution [100 g/l chromium trioxide (CAS no. 1333-82-0) and 25 g/l sodium chloride (CAS no. 7647-14-5)] was applied by dipping for 60 seconds, which is expected to yield a passive film thickness of ~700 nm (13). New trivalent chromium passivation samples (referred to as trivalent 'new' in the following) and hexavalent passivation samples were produced <48 h before the present investigation. Old trivalent chromium passivation samples (referred to as trivalent 'old' in the following) were produced 1 year before this investigation, and stored under ambient conditions in the absence of direct sunlight. The DPC test reagent for hexavalent chromium was produced immediately prior to the experiment by dissolving 0.4 g of 1,5-DPC (CAS no. 140-22-7) in a mixture of 20 ml of acetone (CAS no. 67-64-1) and 20 ml of 96 vol% ethanol (CAS no. 64-17-5), and then adding 20 ml of 75 vol% H₃PO₄ (CAS no. 7664-38-2) and 20 ml of de-ionized water. The solution pH (measured with pH-indicator strips) was 4.

The passivated zinc-plated panels were rinsed with ethanol, dried with pressurized air, and scrubbed against the surface of a cotton bud that was soaked with the DPC test reagent. The visual inspection for an indicative pink–purple colour change (14) was carried out after a 2-min pause. The test was repeated for three samples on both sides of the panels to confirm that results were consistent.

In order to gain information about the surface chemistry of the trivalent 'old' samples and the hexavalent samples, XPS analysis of the specimens was carried out. In addition to allowing chemical composition analysis, the deconvolution of X-ray photoelectron spectra is a powerful way of probing the oxidation state of metal oxides (15). X-ray photoelectron spectra were acquired on a Thermo Scientific K-Alpha⁺, Hvidovre, Denmark instrument fitted with a monochromated Al K-Alpha X-ray source at a photon energy of 1486.7 eV. The Cr2p spectra of all three samples were recorded with 50-eV pass energy under ultrahigh vacuum on a circular area with a diameter of 400 µm by use of a flood gun, and referenced to the adventitious carbon C1s peak at 284.8. The peak deconvolution was performed with the commercial software THERMOAVANTAGE 5.949 with a Shirley-type background. Owing to its insignificant contribution, the asymmetry of the metallic Cr⁰ was neglected, and all edges were fitted as single symmetric peaks.

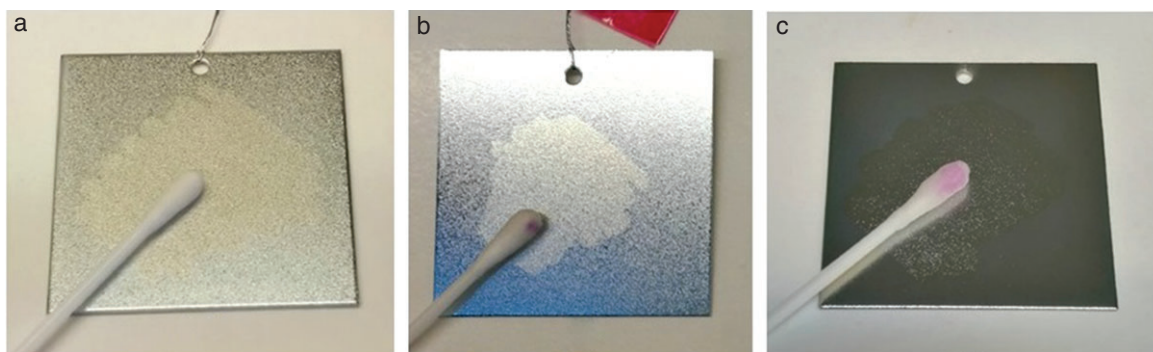


Fig. 1. Diphenylcarbazide spot test for hexavalent ions on **(a)** a trivalent 'new' passivation sample (negative), **(b)** a trivalent 'old' passivation sample (positive), and **(c)** a hexavalent passivation sample (positive). Trivalent 'old' passivation sample acquired a bluish haze because of the ageing.

Results

The DPC spot test on the trivalent 'new' passivation did not show any colouration (Fig. 1a), whereas the test was positive (i.e. indicated a distinct pink colour) for the trivalent 'old' and the hexavalent passivations (Fig. 1b and c).

To verify the presence of hexavalent chromium, XPS was performed on all three types of treatment. High-resolution X-ray photoelectron spectra showing the peak fitting of the Cr 2p_{3/2} feature, acquired on both the trivalent and hexavalent passivation, are shown in Fig. 2. The Cr 2p_{3/2} spectra recorded on the trivalent passivations were deconvoluted into three individual peaks (Fig. 2a and b), whereas the spectrum acquired on the hexavalent passivation was deconvoluted into two individual peaks. The respective peak positions are indicated in Fig. 2.

Discussion

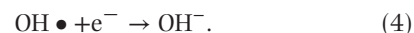
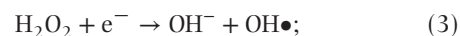
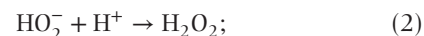
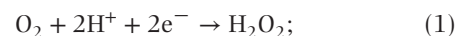
We examined metallic zinc surfaces with (i) freshly produced trivalent chromium passivation, (ii) 1-year-aged trivalent chromium passivation and (iii) new hexavalent chromium passivation for the presence of hexavalent chromium by using two different detection methods. The DPC spot test showed positive test reactions on the latter two surfaces. These results are in agreement with already reported data indicating that the DPC test showed positive test reactions in the trivalent passivation of samples subjected to corrosion testing (10) and samples aged under ambient conditions (11).

The X-ray photoelectron spectrum recorded on the trivalent 'new' sample (Fig. 2a) was deconvoluted into three individual edges, whereby the individual edges were indicative of the presence of trivalent chromium as hydroxide (peak at 577.7 eV) and oxide (peak at 576.6 eV), together with a contribution from metallic

chromium (peak at 574.5 eV). The spectrum recorded on the trivalent 'old' sample (Fig. 2b) was deconvoluted into three individual edges with the positions 577.5, 576.6 and 574.5 eV. In analogy to the trivalent 'old' sample, the features were indicative of the presence of trivalent chromium as hydroxide and oxide, as well as metallic chromium, respectively. Thus, this method could not confirm the presence of hexavalent chromium as suggested by the positive DPC test result. The spectrum recorded on the hexavalent passivation (Fig. 2c), in contrast, was deconvoluted into two single contributions, whereby the high binding energy edge (peak at 579.5 eV) was indicative of hexavalent chromium and the low binding energy feature (peak at 577.3 eV) was indicative of trivalent chromium hydroxide (16).

During the defining reaction of the DPC spot test, hexavalent chromium was reduced by DPC to trivalent chromium, and DPC was oxidized to 1,5-diphenylcarbazone (DPCA) (Fig. 3). The complex compound formed between trivalent chromium and DPCA has a distinctive reddish-purple colour (14, 17). This means that, if the oxidation of DPC to DPCA happens for any other reason than the reduction of hexavalent chromium, and trivalent chromium is present in the reaction zone, a false-positive reaction may be obtained.

It is known that, during atmospheric corrosion, the cathodic reaction of oxygen reduction goes through the following stages (Equations 1–4) (18, 19):



The presence of hydrogen peroxide can induce a Fenton-like reaction (Equation 5) (20), which, in the case

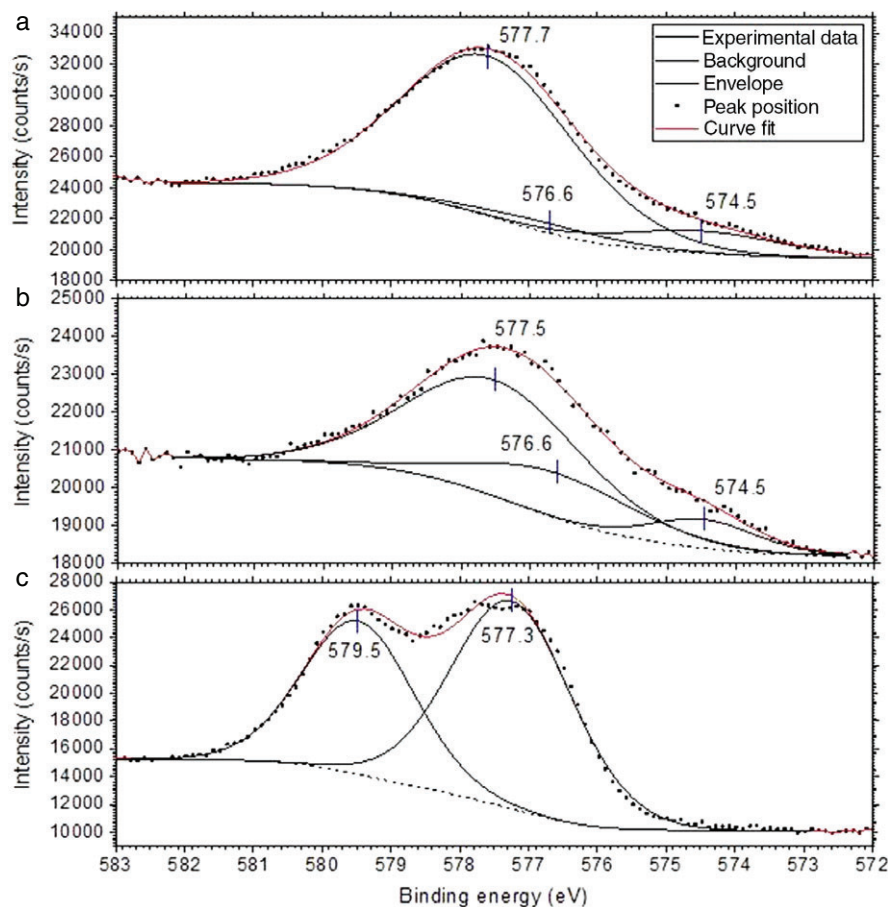


Fig. 2. Deconvolution of the X-ray photoelectron high-resolution spectra of the Cr 2p_{3/2} feature on (a) the trivalent 'new' passivation, (b) the trivalent 'old' passivation, and (c) the hexavalent passivation. The spectra recorded for the trivalent passivations is deconvoluted into edges, owing to chromium hydroxide/oxide and metallic chromium, indicating the absence of hexavalent chromium, whereas the spectrum recorded for the hexavalent passivation shows a high binding energy shoulder, indicating the presence of both trivalent and hexavalent chromium.

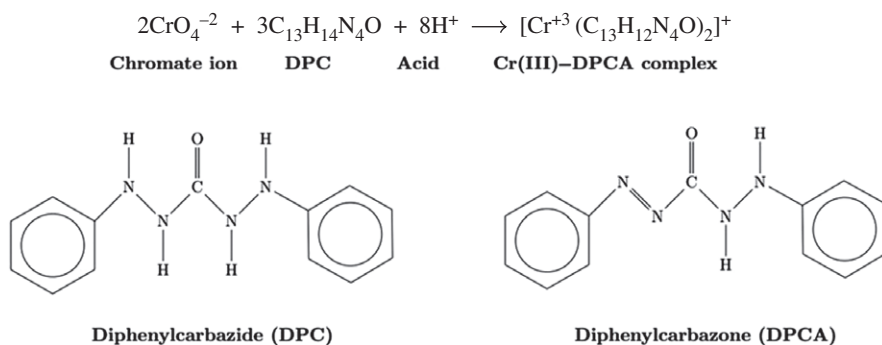


Fig. 3. Reaction between hexavalent chromium and diphenylcarbazine.

of trivalent chromium, is reported to show maximum reactivity and the highest yield of HO• production at neutral pH, but can also occur under acidic conditions (21).



The Fenton process is widely used for the heavy oxidation of organic substances. It is very likely that its oxidation potential can convert DPC into DPCA.

Accordingly, a trivalent 'old' passivation sample is expected to have a higher level of oxidative species

than a trivalent 'new' passivation sample, because of atmospheric corrosion and a Fenton-like reaction with chromium, which can produce highly oxidative hydroxyl radicals. This could explain the positive result of the test on the 'old' trivalent passivation samples and the negative results on the freshly prepared samples. Hexavalent chromium species generated with the Fenton-like process may be further reduced with hydrogen peroxide, which has reducing properties in alkaline media, and was not detected by XPS.

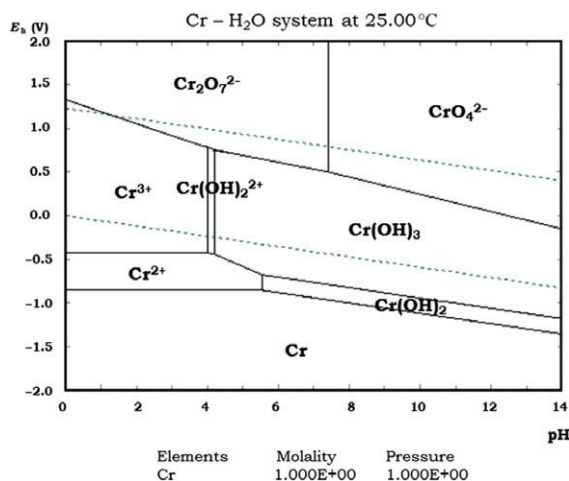
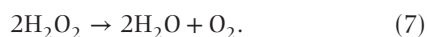
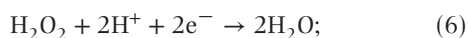


Fig. 4. Pourbaix diagram for the Cr–H₂O system. Calculated with HSC CHEMISTRY[®] 5.1 software.

At the same time, for the DPC reaction, an acidic environment is required, which is achieved by adding phosphoric acid to the testing solution. According to the Pourbaix diagram for Cr–H₂O (Fig. 4), in the lower range of pH passivating Cr(OH)₃ will exist in the form of trivalent ions, supplying a source for the coloured Cr(III)–DPCA complex.

Alternatively, the observations may be explained by the generation of hydrogen peroxide species during the test. As the test reagent is acidic (pH 4), it may initiate active corrosion of the samples. Thus, the cathodic reaction (oxygen reduction) under acidic conditions may also result in intermediate hydrogen peroxide development (Equation 1), and then continues through the following stages (Equations 6 and 7) (22).



Hydrogen peroxide may induce the Fenton-like reaction (Equation 5), as already described above. The more

prominent reaction on the aged trivalent passivated samples than on the freshly prepared ones can be explained by the partial loss of protective properties of the passivation film caused by atmospheric corrosion during storage. This will result in a more active corrosion reaction of the acidic solution on the 'old' sample, generating more oxidative species, and thus a higher degree of oxidation of DPC to DPCA, together with a more distinct colour indication.

Although examples of the practical use of a DPC-based spot test were recently reported (7–9), the test does require further validation. In the present study, we have shown that false-positive test reactions may occur under particular conditions; however, previous results (9) indicate that the test performs well under normal conditions, and that it can be used to reliably detect the release of hexavalent chromium from metal surfaces or leather, if the simultaneous presence of trivalent chromium and a strong oxidizer can be excluded. The DPC test is a screening test and, in particular cases, more accurate methodology is required.

Conclusion

In this study, we identified a false-positive DPC spot test result on trivalent chromium-passivated zinc coatings. Although we cannot explain this observation with certainty, we suggest that unintended oxidation of DPC to DPCA has occurred, leading to a Fenton-like reaction with chromium from a passivating layer that was induced by atmospheric corrosion; however, we emphasize that both hexavalent and trivalent chromium may cause allergic contact dermatitis.

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