

Research Article

Spectrophotometric Quantification of Toxicologically Relevant Concentrations of Chromium(VI) in Cosmetic Pigments and Eyeshadow Using Synthetic Lachrymal Fluid Extraction

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Chromium(VI) salts are possible contaminants of the chromium(III) pigments used as colorants in eyeshadow preparations. The use of products containing these contaminants poses acute risks for sensitization and contact allergies. Chromium(VI) compounds are also classified as carcinogenic to humans (IARC group 1). An analytical method to analyse trace levels of chromium(VI) in eyeshadow was developed in this study. The method is based on an extraction of the chromium(VI) from the sample using a maximum extraction with alkali and additionally with synthetic lachrymal fluid to simulate physiological conditions. Following derivatization with 1,5-diphenylcarbazine, the extracted chromium(VI) is then quantified by spectrophotometry (540 nm). Validation tests indicated a method standard deviation (inter- and intraday) of 8.7% and a linear range up to 25 mg/kg. The average recovery was 107.9%, and the detection limit was 2.7 mg/kg. The applicability of the procedure was confirmed by the analysis of pigments and authentic eyeshadow matrices.

1. Introduction

Eyeshadow preparations can consist of dispersions of the necessary pigments in emulsions, oil, or molten wax. Another possibility is to process the pigments and other ingredients in the form of a pressed powder. The pigment concentration can vary in a wide range between 5 and 70% [1]. According to the EU Cosmetics Directive 76/768/EEC (Annex IV), the only allowable green colorants are chromium hydroxide green ($\text{Cr}_2\text{O}(\text{OH})_4$) and chromium oxide green (Cr_2O_3). However, these chromium(III) pigments can be contaminated with chromium(VI), which may cause an allergic contact dermatitis [2]. Because of its allergenic character, the presence of chromium(VI) is prohibited in cosmetics by a German cosmetics regulation and also by the corresponding EU Directive 76/768/EWG.

Contact allergies caused by chromium(VI) are generally known from reactions to chromium(VI) in cement or leather. Chromium(VI) can also be found in other consumer goods, particularly in green soaps and detergents [3]. However, only low levels of chromium(VI) are typically

contained in cosmetics, and are not expected to lead to an initial sensitization. However, if a consumer has already been sensitized to chromium(VI) from other sources, the low concentrations in leave-on cosmetics could nevertheless be allergenic [4]. In this respect, the product that possesses the highest inherent risk is eyeshadow, because it may contain up to 70% of green chromium(III) pigments.

The “Informationsverbund Dermatologischer Kliniken zur Erfassung und wissenschaftlichen Auswertung von Kontaktallergien (IVDK),” a German institution for the collection and assessment of clinical data concerning severe contact allergies, estimates that 0.3% of the German population is sensitive to chromium(VI) [5]. In addition to their allergenic effect, chromium(VI) compounds may cause chronic toxicity and they have been classified as carcinogenic to humans (IARC group 1) [6]. Therefore, for at least a subgroup of the consumer population, chromium(VI) may potentially be harmful to human health.

According to the EU Cosmetics Regulation EC/1223/2009, Article 17, chromium(VI) may be tolerated in technically unavoidable traces only if these traces are evaluated as

safe in the final product. In the past, no adequate method was available for the control of chromium(VI) at the lower mg/kg levels in eyeshadow. Therefore, a threshold for these technically unavoidable traces has not yet been established because of lack of data. The German Federal Institute for Risk Assessment (BfR) holds the position that 1 mg/kg chromium(VI) in cosmetics could lead to contact dermatitis [7]. In contrast, Basketter et al. [4] estimated that a chromium(VI) content of less than 5 mg/kg in consumer products would be considered safe. In the present study, a method was developed and validated for the measurement of chromium(VI) in the range of 1–5 mg/kg in eyeshadow and eyeshadow pigments. The extraction parameters were optimized with respect to their influence on the measurements of chromium(VI) content. We used spectrophotometry because this method was simpler and less expensive than previously developed methods based on ion chromatography [8]. We also evaluated the stability of the extracted chromium solutions and of the 1,5-diphenylcarbazide (DPC) complex used for spectrophotometric measurement.

2. Materials and Methods

2.1. General Method Description. In brief, water-soluble chromium(VI) was extracted by suspending the sample in an aqueous medium. An aliquot of the extraction solution was acidified, and a solution of DPC was added. A redox reaction caused a reduction of chromium(VI) to chromium(III), with concomitant oxidation of DPC to 1,5-diphenylcarbazone (DPCA). The DPCA and chromium(III) formed a magenta-coloured complex with an absorption maximum of 540 nm (for details on this mechanism, see [9–13]). This complex was measured with a spectrophotometer against a water blank. External calibration was used for quantification. Influencing factors were evaluated by varying the parameters of the extraction and detection.

2.2. Chemicals and Solutions. Phosphoric acid solution was prepared by making 70 mL H_3PO_4 (85%) to 100 mL with distilled water in a graduated flask. An alkaline solution was prepared by dissolving 10 g NaOH and 30 g Na_2CO_3 in distilled water in a 1000 mL graduated flask. After adjustment to pH 11.5 with phosphoric acid solution, the solution was made up to 1000 mL with distilled water. Synthetic lachrymal fluid consisted of a mixture containing 4.3 g NaCl, 8.7 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.69 g NaH_2PO_4 , and 974.2 g distilled water. DPC solution was prepared by combining 0.5 g DPC and 1 drop of glacial acetic acid in a 50 mL graduated flask and making the volume to 50 mL with acetone.

Chromium stock solution consisted of 0.2829 g dried $\text{K}_2\text{Cr}_2\text{O}_7$ made up to 100 mL with distilled water in a graduated flask. Chromium standard solution was prepared by adding 1 mL of the chromium stock solution to a graduated flask and adjusting the volume to 1000 mL with distilled water.

2.3. Calibration. Calibration was performed using diluted chromium standard solution at concentrations of 0.1–4.0 μg

Cr(VI)/10 mL. After a filtration through a 0.2 μm membrane filter, 5 mL of the calibration solution was used for the colour reaction.

2.4. Colour Reaction. A 5 mL aliquot of the calibration standard or the sample solution was combined with 1 mL of the DPC solution and 1 mL of a 70% H_3PO_4 solution and the volume was made up to 10 mL with water. After 30 min, the colour intensity was measured spectrophotometrically at 540 nm against a water blank.

2.5. Extraction under Physiological Conditions. Synthetic lachrymal fluid, pH 7.0, was used for extraction to simulate physiological conditions. The sample was agitated for 45 min at 300 rpm and 95°C in 40 mL of synthetic lachrymal fluid, followed by a 30 min cooling-down period, with agitation. The sample was then quantitatively transferred into a 50 mL volumetric flask and made up to 50 mL with distilled water. The sample was filtered through a 0.2 μm membrane filter, and the filtered solution was used for the colour reaction.

2.6. Maximum Extraction Procedure. Extraction of the maximum of chromium(VI) from the sample required optimization of several extraction parameters, including working with strongly alkaline solutions and high temperatures. The sample was agitated for 3 hours at 300 rpm and 70°C in 40 mL of the alkaline solution. After 30 min of agitated cooling, the sample was filtered through a 0.2 μm membrane filter and the filtered solution was used for the colour reaction.

3. Results and Discussion

3.1. Optimization of Extraction. Initial experiments used physiological extraction conditions of 37°C and 14 hours to simulate realistic eyeshadow application conditions. In subsequent trials, the extraction conditions were modified by reducing the time and raising the temperature (see Section 2.5). This had the advantage of significantly increasing the sample throughput, and our trials showed no significant differences in the measurement results.

The quantification of chromium(VI) was dependent on several extraction parameters. The chromium(VI) content in the final extract was linearly dependent on the temperature (coefficient of correlation = 0.9925). As shown in Figure 1, increases in the pH of the extraction solution resulted in an exponential rather than a simple linear increase in the amount of chromium extracted (coefficient of correlation = 0.9829).

Increases in the extraction volume resulted in a quadratic increase in the extraction (coefficient of correlation = 0.9567), while increases in the extraction time showed logarithmic increases in extraction (coefficient of correlation = 0.9893). Similar effects were not observed for Cr_2O_3 (i.e., when the pure chemical was tested as a reference substance). A possible confounding effect due to oxidation of chromium(III) to chromium(VI) was not likely to explain

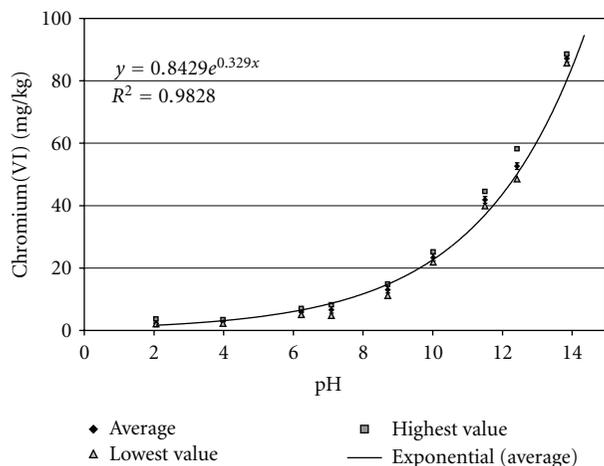


FIGURE 1: pH dependence of the chromium(VI) concentration.

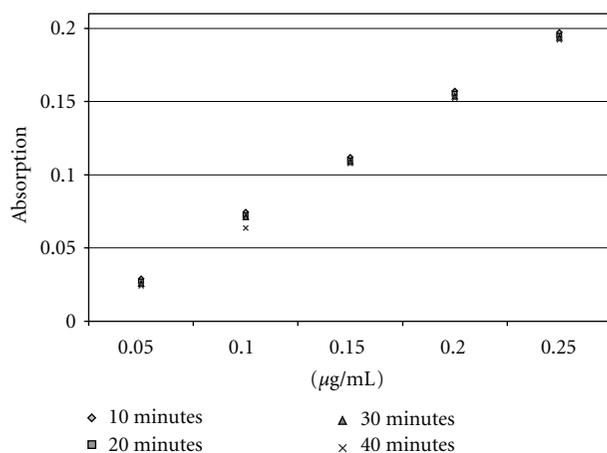


FIGURE 2: Stability of the DPCA-Cr(III) complex.

these effects; rather, they appeared to depend solely on the physicochemical influences of the extraction medium.

3.2. Optimization of Detection and Discussion of Interferences.

Tests were made to confirm the stability of the DPC-chromium complex. After the minimum time of 15 min necessary for the formation of the complex, the reaction product was stable for at least 40 min. None of the samples showed any changes in absorption (Figure 2) indicating that the DPC-chromium complex was stable regardless of the sample matrix. This result confirms previous findings in the literature [2]. The concentration of the DPC solution also had no influence on the test result (i.e., if DPC was present in excess).

Previously published literature indicates that this assay can be subject to interference by Cu(II), Mo(VI), Hg(I/II), and V(V) [14]. Of these, vanadium is especially relevant as it may form complexes with DPC that have a similar absorption maximum to the chromium complex. However, these vanadium complexes are unstable and decolorize

TABLE 1: Detection and quantification limits.

	Detection limit	Quantification limit
Sample solution	0.4 µg/10 mL	0.7 µg/10 mL
Matrix	2.7 mg/kg	4.7 mg/kg

quickly, in less than 15 min [2]. For this reason, it was also necessary to wait at least 15 min before conducting measurements. If necessary, very high amounts of vanadium can also be removed using 8-hydroquinoline at pH 4 [13]. In the current study, all samples were first screened by ICP-MS to exclude the presence of interfering elements.

3.3. Validation. Both extraction methods could be used for the quantification of chromium(VI) in cosmetic pigments. The validation of the method with synthetic lachrymal fluid led to a method standard deviation (inter- and intraday) of 8.7% and a linear range up to 25 mg/kg. A standard deviation in this range is judged as sufficient for the purpose of the method according to the criteria of Horwitz [15]. The average recovery was 104.7%. Calibration in the matrix and an external calibration yielded similar results, indicating that an external calibration was acceptable for routine analysis. No constant or proportional systematic errors were encountered. The extracted sample solution was stable for at least 4 days.

The maximum extraction procedure was validated resulting in a method standard deviation (inter- and intraday) of 9.2% and a linear range up to 125 mg/kg. The performance was similarly judged as acceptable [15]. The average recovery was 107.9%. However, in this case, comparison of internal and external calibration showed constant and proportional systematic errors and the slopes of the calibration curves were significantly different. Therefore, use of an internal calibration (standard addition) is necessary. The extracted sample solution is not stable for 5 days; hence, the sample extract should be measured immediately after extraction.

The detection and quantification limits for both methods are shown in Table 1. The limits either refer to the volume used for the colour reaction or were calculated as mg Cr(VI)/kg sample for a sample weight of 1.5 g.

3.4. Application of the Method for Authentic Eyeshadow Products.

The described extraction methods could be used for the quantification of chromium(VI) in the cosmetic pigments (raw materials) that were used in the method development. The next step was to transfer these methods to real eyeshadow matrices. A defatting step was needed prior to measuring the chromium(VI) content of eyeshadow, but the type of defatting solvent used had no influence on the extraction yield (Figure 3). Hexane was used for subsequent experiments. Ultrasonic homogenisation also had no influence on the extraction yield.

The method for eyeshadow was evaluated for two different self-prepared eyeshadows. The first one was based on

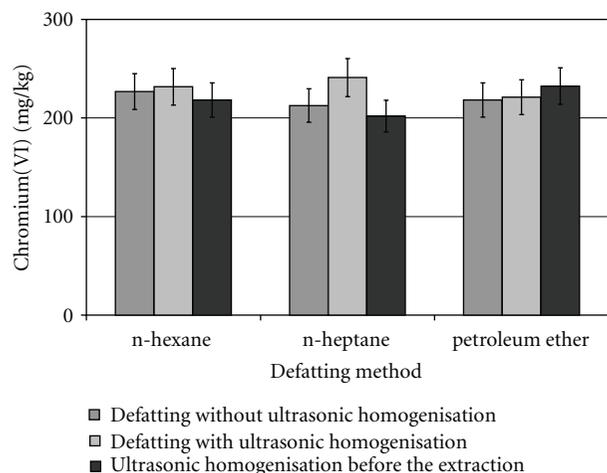


FIGURE 3: Influence of defatting on extraction yield.

a contaminated pigment with a known chromium(VI) content. The second one was produced from a chromium(VI)-free pigment by spiking it with a defined amount of potassium dichromate. Only 20% of the estimated chromium(VI) content of the first eyeshadow was recovered during analysis. The potassium dichromate was completely recovered in all spiked samples, independent of the extraction conditions. This confirms that chromium(VI) was unlikely to have been reduced to chromium(III) during the cosmetics production process. The cause of the effect in contaminated pigment is unknown. Most likely, interferences of the sample matrix or changes in the pigment structure during the production process are responsible for the low recovery of 20%. Chromium(VI) impurities are possibly bound within chromium(III) oxide crystals. High mechanical forces such as homogenizing could also lead to a change in the crystal structure, which might lead to a reduced solubility of chromium(VI).

In a third experiment, a defined amount of pigment was added to the first eyeshadow to exclude interferences within the sample matrix. The recovery of added pigment to contaminated eyeshadow samples (i.e., after the production process) was high (70–90%). Recovery of potassium dichromate added to eyeshadows was also good (80–98%). Therefore, interferences within the sample matrix and reduction during the production process or the analysis could be excluded as reasons for the observed low recovery. Changes in the crystal structure of the pigment most likely explain the low recovery. This assumption has to be confirmed by further work, for example, by crystallographic research.

4. Conclusions

The extraction of commercial eyeshadow preparations under physiological conditions by a single procedure does not provide sufficient analytical safety to determine toxicologically relevant trace concentrations of chromium(VI) (i.e., in the range of 1–5 mg/kg). This is due to the very low recoveries of chromium when analyzing eyeshadow, which we currently cannot fully explain. Therefore, a combination

of different sample preparation methods is needed to analyse the chromium(VI) content in eyeshadow preparations.

As a first step, the eyeshadow has to be analysed following a maximum extraction procedure, as described here. If this leads to an absorption value above 0.02, the sample can be considered as qualitatively chromium(VI) positive. This will trigger an analysis of the pigment raw material that was used to produce the commercial product. The pigment again should be analysed following extraction by the maximum extraction procedure. If it yields a positive result (based on our experience, more than 30 mg/kg in the raw material will result in detectable levels in the final eyeshadow product), it should then be analysed using the lachrymal fluid method, which can verify if an actual risk for the consumer exists under simulated physiological conditions. If the lachrymal fluid is also positive, the product should be judged as a serious risk to the consumer. The raw material should then be prohibited for use in the production of eyeshadow, as stipulated by the EU Cosmetics Directive (Annex IV).

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