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Chemical research on red pigments after adverse reactions to tattoo

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Summary
Currently, the incidence of tattooing is on the rise compared to the past, especially among adolescents, and it leads to the urgency of monitoring the security status of tattooing centers, as well as to inform people about the risks of tattoo practice. In our clinical experience, 20% of tattooed patients presented adverse reactions, like allergic contact dermatitis, psoriasis with Koebner's phenomena and granulomatous reactions, with the latter most prevalent and most often related to red pigment. Adverse reactions to tattoo pigments, especially the red one, are well known and described in literature. Great attention has to be focused on the pigments used, especially for the presence of new substances, often not well known.
For this reason, we decided to perform a study on 12 samples of red tattoo ink, obtained by patients affected by different cutaneous reactions in the site of tattoo, to analyze their chemical composition.

Introduction

The practice of tattooing is very common worldwide: more than 24% of American adults have one or more tattoos, with increasing interest and popularity also in Italy. The art of tattooing has ancient origins and was gradually linked to specific meanings like religious beliefs, tribal affiliation, loyalty to a leader, courage, therapeutic functions. Actually, the incidence of tattooing is on the rise compared to the past, especially among adolescents, and it leads to the urgency of monitoring the security status of tattooing centers, as well as to inform the people about the risks of tattoo practice (1-4).
The process of tattooing involves the repetitive piercing of the skin with ink-filled needles, with possible local or systemic complications, classified by different authors in allergic, inflammatory, infectious and neoplastic (5-7).
In our clinical experience, 20% of tattooed patients presented adverse reactions, like allergic contact dermatitis, psoriasis with Koebner's phenomena and granulomatous reactions, with the latter most prevalent and most often related to red pigment. Adverse reactions to tattoo pigments, especially the red one, are well known and described in literature (8,9).
Great attention has to be focused on the pigments used, especially for the presence of new substances, often not well known. For this reason, we decided to perform a study on 12 samples of red tattoo ink, obtained by patients affected by different cutaneous reactions in the site of tattoo, to analyze their chemical composition.

Material and Methods

The ink samples under study were labeled with nomenclature from TIR1 to TIR12 by order of arrival in laboratory, but especially because in most of them the exact chemical composition was not described.

Chemicals, Reagents and Solutions. Methanol for analysis and HPLC grade solvents were purchased from Sigma-Aldrich (Milan, Italy) and Carlo Erba (Milan, Italy). Detailed information on the analysed samples, i.e. producers, production conditions, storage method, etc., can be obtained by directly asking the correspondence author.
Chromatographic equipment. The HPTLC system (CAMAG, Muttenz, Switzerland) (10-12) consisted of Linomat 5 sample applicator using 100 mL syringes and connected to a nitrogen tank; chamber ADC 2 containing twin trough chamber 20 x 10 cm; Immersion device III; TLC Plate Heater III; TLC visualizer linked to winCATS software. Glass plates 20 x 10 cm (Merck, Darmstadt, Germany) with glass-backed layers silica gel 60 (2 µm thickness). Before use, plates were prewashed with methanol and dried for 3 min at 100 °C.
Sample preparation and application. The samples (5 µL each) were dissolved in water (1000 µL). Solutions were applied with nitrogen flow. The operating conditions were: syringe delivery speed, 10 s µL-1 (100 nL s-1); injection volume, 4 µL; band width, 8 mm; distance from bottom, 15 mm.
Development and derivatisation. The HPTLC plates were developed in the automatic and reproducibly developing chamber ADC 2, saturated with the same mobile phase, dichloromethane / methanol 9:1 (v/v), for 20 min at room temperature. The developing solvents (i.e. type of solvents and ratios) were carefully optimised before the analyses. The length of the chromatogram run was 70 mm from the point of application. The developed layers were allowed to dry on TLC Plate Heater III for 5 min at 120 °C and then derivatised with a selected solution, including anhysaldehyde (170 ml methanol, 20 ml acetic acid, 10 ml sulfuric acid, 1.00 ml anisaldehyde). Finally, the plates are warmed for 5 min at 120 °C before inspection.
Inspection. All treated plates were then inspected under a UV light at 254 or 366 nm or under reflectance and transmission white light (WRT), respectively, at a Camag TLC visualiser, before and after derivatisation.
Documentation. CAMAG DigiStore2 digital system with winCATS software 1.4.3 was used for the documentation of derivatised plates.
Stability and Validation. Sample solution of the ink were prepared and stored at room temperature for 3 days and then applied on the same HPTLC plate and the chromatogram evaluated for additional band. Similarly band stability was checked by keeping the resolved peaks and inspecting at intervals of 12, 24 and 49 h. Overlapping of bands is a typical analytical challenge for complex mixtures like multi-ingredient products. HPTLC allowed a good separation and visualisation of the constituents. Sample solutions of the extracts were found to be stable at 4 °C for at least 1 month and for at least 3 days on the HPTLC plates. Repeatability was determined by running a minimum of three analyses. RF values for main selected compounds varied ± 0.02 %. The effects of small changes in the mobile phase composition, mobile phase volume, duration of saturation were minute and reduced by the direct comparison. On the contrary, the results were critically dependent on prewashing of HPTLC plates with methanol.

Figure 1 *- HPTLC fingerprint analysis of Tattoo Ink. Mobile phase: Dichloromethane / Methanol (9:1 v/v). Visualisation: 254 nm. Before derivatisation. Tracks: 1, TIR1; 2, TIR2; 3, TIR3; 4, TIR4; 5, TIR5; 6, TIR6; 7, TIR7; 8, TIR8; 9, TIR9; 10, TIR10; 11, TIR11; 12, TIR12.*

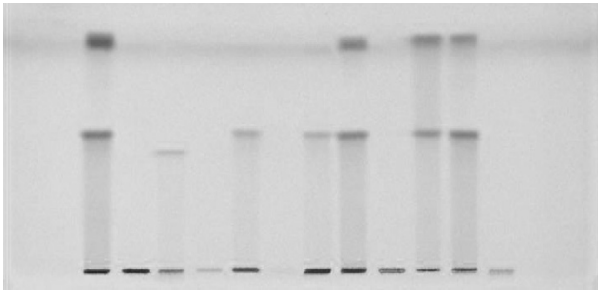


Figure 2 *- HPTLC fingerprint analysis of Tattoo Ink. Mobile phase: Dichloromethane / Methanol (9:1 v/v). Visualisation: 366 nm. Before derivatisation. Tracks: 1, TIR1; 2, TIR2; 3, TIR3; 4, TIR4; 5, TIR5; 6, TIR6; 7, TIR7; 8, TIR8; 9, TIR9; 10, TIR10; 11, TIR11; 12, TIR12.*

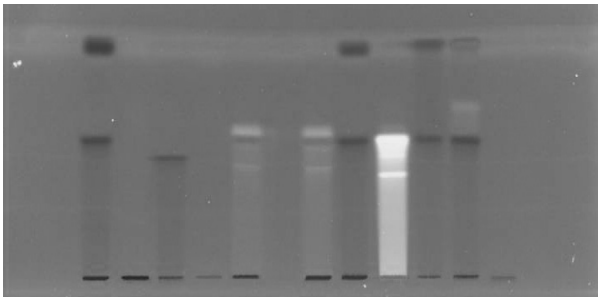


Figure 3 *- HPTLC fingerprint analysis of Tattoo Ink. Mobile phase: Dichloromethane / Methanol (9:1 v/v). Visualisation: white light. Derivatisation: anhysaldehyde. Tracks: 1, TIR1; 2, TIR2; 3, TIR3; 4, TIR4; 5, TIR5; 6, TIR6; 7, TIR7; 8, TIR8; 9, TIR9; 10, TIR10; 11, TIR11; 12, TIR12.*

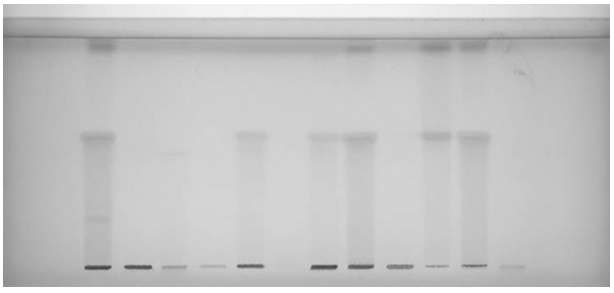
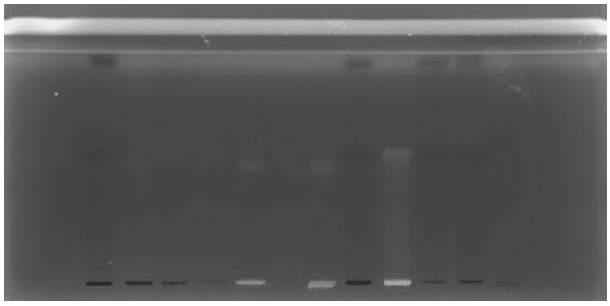


Figure 4 *- HPTLC fingerprint analysis of Tattoo Ink. Mobile phase: Dichloromethane / Methanol (9:1 v/v). Visualisation: 366 nm. Derivatisation: anhysaldehyde. Tracks: 1, TIR1; 2, TIR2; 3, TIR3; 4, TIR4; 5, TIR5; 6, TIR6; 7, TIR7; 8, TIR8; 9, TIR9; 10, TIR10; 11, TIR11; 12, TIR12.*



Results and discussion

The study identified two groups of inks: the first one consists of samples TIR1, TIR8 and TIR10, while TIR11 presents two components more; the second one includes samples TIR5, TIR7 and TIR9, very similar. However, TIR5 and TIR9 present a common spot to the other samples.

The proofs concerning solubility showed several different groups: in the first one, sample TIR2 results vey soluble in water (probably due to the presence of polar compounds). The second group includes samples TIR5, TIR6 and TIR12, which are slightly soluble / insoluble in water and in other solvents. This fact made it impossible to obtain a chromatogram.

In order to have a precise and complete profile, the HPTLC plates were read at different wavelengths (UV 254 and 366 nm, white light WRT), before and after derivatisation with anisaldehyde-sulfuric acid.

The data obtained show that only in few cases the samples have similar fingerprints. This may be due to the use of different pigments for the formulation of various red shades.

Further analyses not listed in this study as in progress investigation, reveal the presence of toxic substances in some inks tested. This fact lets us hypothesize a link between the inks used for tattooing and the different skin reactions often observed in the areas of tattoos.

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