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Tattoo Pigments are Cleaved by Laser Light—The Chemical Analysis *In Vitro* Provide Evidence for Hazardous Compounds¶

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ABSTRACT

In the western world, more than 80 million people decorate their skin with tattoos. Tattoo colorants are injected into the skin, like medical drugs. Most tattoo colorants are industrial pigments, and chemical industries have never produced them for human use but only to stain consumer goods. Up to 10% of tattooed people request removal of their tattoos because of an improved self-image or social stigmatization. In contrast to tattooing, physicians usually perform the tattoo removal. For that purpose laser light at very high intensities irradiates the skin to destroy the tattoo pigments. Based on a recent analysis of tattoo pigments, two widely used azo compounds were irradiated in suspension with laser and subsequently analyzed by using quantitative high-performance liquid chromatography and mass spectrometry. The high laser intensities cleaved the azo compounds, leading to an increase of decomposition products such as 2-methyl-5-nitroaniline, 2-5-dichloraniline and 4-nitro-toluene, which are toxic or even carcinogenic compounds. Moreover, the results of the chemical analysis show that the tattoo colorants already contain such compounds before laser irradiation. Because of a high number of patients undergoing laser treatment of tattoos and based on the results of our findings *in vitro*, it is an important goal to perform a risk assessment in humans regarding laser-induced decomposition products.

INTRODUCTION

In the western world, tattooing seems to be a sign of self-destructive and rebellious behavior (1). Millions of people have at least one decorative tattoo. Cosmetic tattoos, to mimic eye, lip or eyebrow liner, have also become increasingly popular (2).

In the past, coloring agents were inorganic pigments, whereas for dark blue amateur tattoos, commercially available ink is still in use. Because tattoo compounds in comparison with cosmetics are not officially controlled, the origin and chemical structure of these coloring agents are hardly known. Consequently, neither the tattoo artist nor the tattooed patient has any information about the compounds punctured into the skin.

Recently, an extensive analysis of a large number of tattoo compounds was performed for the first time (3). Most of the commercially available tattoo compounds are organic pigments classified by their chemical constitution (4).

Generally, the tattoo pigments are well tolerated by the skin. Nevertheless, adverse reactions have been published in the literature (5–8). Moreover, several malignant lesions have occurred in tattoos (maybe coincidental) (9–11). Because of an improved self-image or social stigmatization, a significant number of people undergo a therapy of tattoo removal by using predominantly Q-switch lasers. Most of the tattoo pigment is found within cells and not free within the dermis. Although many pigment particles measure "a few microns," others are significantly larger (12) or when accumulated within cells may act as larger aggregate bodies. According to the principles of selective photothermolysis (13), the laser impulses show a high intensity and ultrashort pulse durations of a few nanoseconds (Q-switched lasers). The laser pulses change the shape and the size of the tattoo particles abruptly as proved by histology (12).

However, the exact mechanisms of action regarding the destruction of tattoo pigments are still unclear. After being absorbed in the pigment molecule, the energy of the laser light is converted to heat or breaks chemical bonds inside the molecule. The ultrashort heating (*ca* in ns) of the pigment may lead to disruption of the pigment. At the same time, the extremely hot surface of the pigment leads to a rapid expansion of the surrounding water, inducing negative pressure and a shock wave near the surface of the pigment. As demonstrated for a suspension of small particles in water, these shock waves may help destroy the tattooed compounds (14).

As a response, a multitude of mechanisms may occur at the same time. Particles pulverize and form a solution of pigment molecules. Molecules can break up, resulting in decomposition products or molecular structure change. Because of fragmentation of the tattoo particles, the skin releases the small pigment particles, unknown decomposition products and newly generated chemical compounds through the lymphatic system. All these mechanisms induce a decrease of the color strength of the pigments responsible for a noticeable clearance of a tattoo.

There is no clinical approval of the tattoo pigments punctured into the skin (15,16), and there are no investigations regarding the

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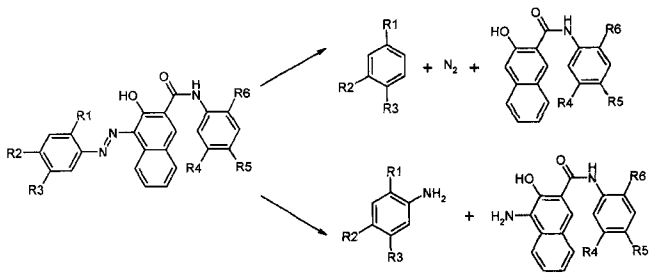
Abbreviations: CR, Cardinal Red; 2,5-DCA, 2-5-dichloroaniline; 1,4-DCB, 1-4-dichlorobenzene; HPLC, high-pressure liquid chromatography; 2-MNA, 2-methyl-5-nitroaniline; 4-NT, 4-nitrotoluene.



R1	R2	R3	R4	R5	R6	name
Cl	H	Cl	H	H	OCH_3	I8
CH_3	H	NO_2	H	H	H	Cardinal Red

Figure 1. The chemical structure of P.R.22 and P.R.9 used as coloring pigments in Cardinal Red (CR) and I8, respectively. For both pigments, the possible decomposition pattern is shown. Additional change of the decomposition products is possible (chlorine, oxidation). The substituents of the pigment molecules are listed in the table inside the figure.

decomposition products induced by laser therapy of tattoos, so far. Because of the numerous patients treated with those laser systems, it is desired and necessary to investigate the decomposition products of tattoo pigments induced by high laser intensities.

The major goal of this investigation was to make the first quantitative analysis of tattoo pigments after laser irradiation by means of high-pressure liquid chromatography (HPLC) and mass spectrometry. For this investigation, the red pigment "Cardinal Red" (CR) and I8 were used exemplarily. Both are widespread tattoo pigments analyzed previously (3). It is well known, that red pigments cause many allergic reactions (7), yet without laser irradiation. CR and I8 are monoazo pigments, whose azo groups cleave either by thermal energy or even in the electronically excited state after light absorption. It is well known that an increase of temperature in azo dyes above 280°C forms 3,3′-dichlorobenzidine (17), a proven carcinogen of human lymphocytes (18). The laser irradiation should cause a higher temperature in such compounds as compared with 280°C.

MATERIAL AND METHODS

CR (P.R.22, color index no. C.I.12315) and I8 (P.R.9, color index no. C.I.12460) are monoazo pigments that were purchased from National Tattoo Supply, Allentown, PA (3). Before irradiation, 2.3 mg CR or I8 was suspended in 0.3 mL acetonitrile (8143 HPLC Gradient Grade, Mallinckrodt Baker, Griesheim, Germany) and filled in a glass reaction vessel (supelco micro 33295, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany).

A frequency-doubled Nd:YAG laser (Wavelight, Erlangen, Germany) at a wavelength of 532 nm, which is absorbed in CR or I8, was used. The overall volume of the pigment suspensions was irradiated with light impulses of 15 mJ, a pulse duration of 8 ns, a repetition rate of 10 Hz for 10 min, leading to a total light dose of 90 J. The spot size was 1 mm, yielding a fluence of 2 J/cm^2.

After laser irradiation the suspension was filtered using a PTFE filter (Chromafil, O-20/15, organic, size of the pore 0.2 µm, Macherey-Nagel, Düren, Germany) and 100 µL diethylenglykol-dimethylether (Diglyme) (32209 Fluka Chemie AG, Deisenhofen, Germany) were added. The concentration of the filtered, clear solution was increased up to 100 µL by stirring and flowing nitrogen gas (0.2 bar, 3–4 min.). After that, the solution was fed into the modular HPLC system (Hewlett-Packard GmbH, Waldbronn, Germany). The system consists of a HP 1050 Quaternary Pump, model no. 79852AX; HP 1050 Autosampler, model no. 79855A; HP 4 Channel Online Degasser, model no. G1303AX; and an Agilent 1100 Photo Diode Array Detector, model no. G1315b (Agilent Technologies GmbH, Waldbronn, Germany). A HPLC-3D-ChemStation Rev. A.08.03 was used for data analysis. The analytical column used was a Synergi Max RP 12 (150 × 2.0 mm internal diameter, 4 µm particle size) from Phenomenex (Aschaffenburg, Germany). Gradient elution was done with water using 0.0059% (wt/vol) trifluoro acetic acid (solvent A) and acetonitrile (solvent B) at a constant flow rate of 400 µL/min. A gradient profile with the following proportions of solvent B was applied (t [min],%B): (0,10), (40,48), (60,98), (70,98). The compounds described were monitored at 258 nm. The injection volume was 10 µL.

The concentrations of 2-methyl-5-nitroaniline (2-MNA), 4-nitrotoluene (4-NT), 2-5-dichloroaniline (2,5-DCA), 1-4-dichlorobenzene (1,4-DCB) (Merck KGaA, Darmstadt, Germany) in the solutions were determined by the method of internal standard. For each compound (i), the calibration factor (CF_i) was determined in a calibration run (single-level calibration). The respective concentration of the standard was chosen to be in the range of the concentration of the decomposition product

$$CF_i = \frac{f_{Tr}}{f_i} = \frac{m_i^K \cdot a_{Tr}^K}{m_{Tr}^K \cdot a_i^K}$$

where f_{Tr} is the response factor of the tracer, m_i^K the mass of compound i in the solution k and m_{Tr}^K the mass of tracer solution k. a_{Tr}^K is the area of tracer in solution k and a_i^K the area of compound i in solution k.

A triple stage mass spectrometer (TSQ 7000, Thermoquest Finnigan, Toronto, Canada) was used to determine the respective mass of the chemical compounds, particularly of the laser-induced products.

To heat up the pigments the suspensions were filled into a glass reaction vessel (supelco micro 33295, Sigma-Aldrich Chemie GmbH) and kept in a oil bath at temperatures of 50°C, 100°C, 150°C or 200°C for 30 min.

RESULTS

The decomposition pattern of CR and I8 is shown in Fig. 1. The absorbed laser light leads to the cleavage of the azo group of the pigment molecules. As a result, 2-MNA, 4-NT, 2,5-DCA, 1,4-DCB, naphthol-AS or methoxy-naphthol-AS should appear in the suspension. The quantification of these compounds was performed by chromatography before and after laser irradiation. Therefore, the chromatography was calibrated for 2-MNA, 4-NT, 2,5-DCA and 1,4-DCB.

At first, the concentrations of these decomposition products were determined before irradiation (Figs. 2a and 3a). The concentrations were 1.6 ± 0.3 µg/mL (2-MNA), 1.0 ± 0.2 µg/mL (4-NT) and 11.8 ± 0.3 µg/mL (2,5-DCA), whereas the concentration of 1,4-DCB was below the detection limit of the system used. Next, the irradiation of the pure solvent showed no effect.

After the laser irradiation of the tattoo pigments, the concentration of the decomposition products increased significantly (Figs. 2b and 3b). When using CR, the concentration of 2-MNA or 4-NT increased 33-fold or 45-fold, respectively. With I8, the concentration of 2,5-DCA or 1,4-DCB increased 7-fold or 33-fold, respectively (Table 1). Mass spectrometry confirmed the identity of these decomposition compounds.

In addition, the UV–VIS spectra (Fig. 4a–e) showed an excellent correlation of the decomposition products and the respective standards for 2-MNA, 4-NT 2,5-DCA, 1,4-DCB and naphthol-AS. Because methoxy-naphthol-AS was not available as a pure substance, the mass spectrum is given for that compound in Fig. 4f. As shown in the Figs. 2 and 3, many other products appear in the suspension after laser irradiation remaining unidentified, so far.

The peak of CR in Fig. 2b (after laser irradiation) appears higher than the respective peak in Fig. 2a. It seems that there is at least one further decomposition product (after laser irradiation) hidden in the CR Peak at 56.1 min (Fig. 2b), which is not obvious at the HPLC wavelength of 258 nm. However, a double peak appeared in the

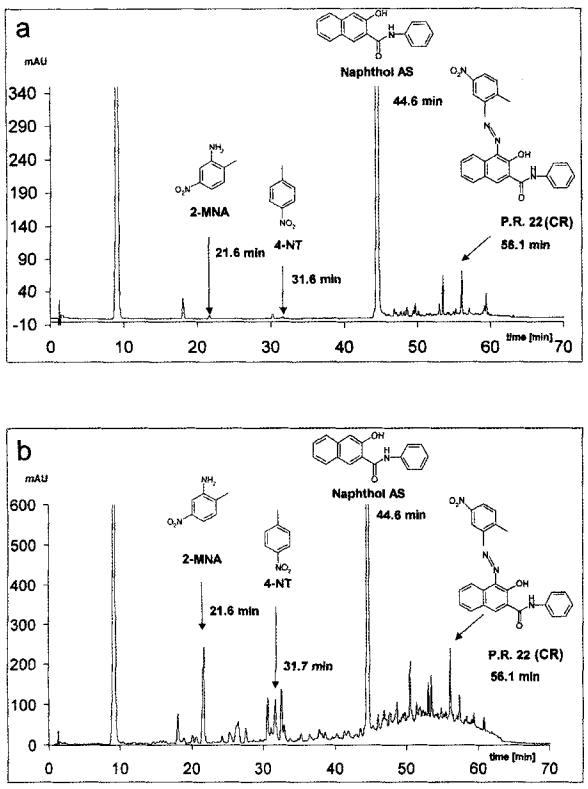


Figure 2. The chromatogram of Cardinal Red (CR) before (a) and after (b) laser irradiation. Every compound fed into the HPLC needs a certain time (min) to appear at the detector. Every peak corresponds to a different compound. The first peak (without any description) is the tracer used for HPLC. To achieve better illustration of the data, different scales are used for the intensity of HPLC detection (mAU). The chemical formulae of the coloring pigment P.R.22 and of the decomposition products, such as 2-methyl-5-nitroaniline (2-MNA), 4-nitrotoluene (4-NT) and naphthol-AS, are included in the diagram.

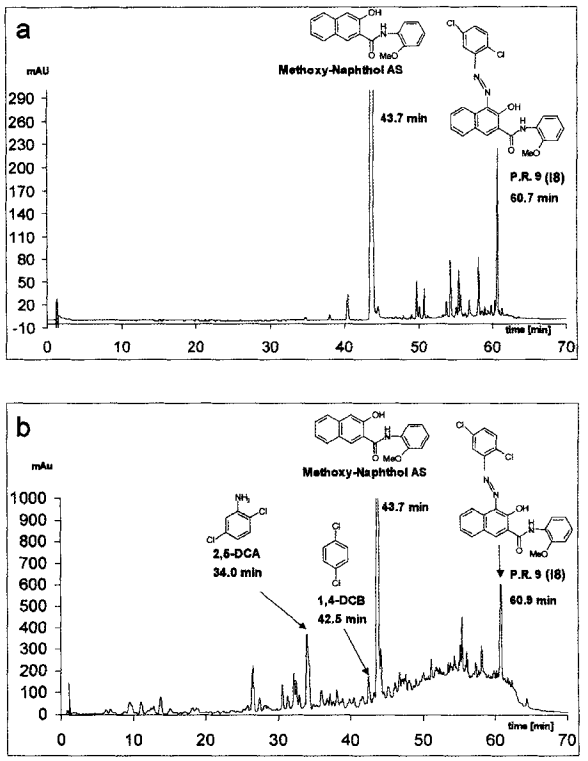


Figure 3. The chromatogram of I8 before (a) and after (b) laser irradiation. Every compound fed into the HPLC needs a certain time (min) to appear at the detector. Every peak corresponds to a different compound. The first peak (without any description) is the tracer used for HPLC. To achieve better illustration of the data different scales are used for the intensity of HPLC detection (mAU). The chemical formulae of the coloring pigment P.R.9 and of the decomposition products, such as 2-5-dichloroaniline (2,5-DCA), 1-4-dichlorobenzene (1,4-DCB) and methoxy-naphthol-AS, are included in the diagram.

HPLC isoplot at 558 nm, which was not present before laser irradiation (data not shown).

The better solubility of naphthol-AS and methoxy-naphthol-AS in the solvent used leads to HPLC peaks higher as compared with the pigment red or I8 peaks. The wavelength of the laser (532 nm) is well absorbed in the pigments but not in naphthol-AS or methoxy-naphthol-AS. The corresponding spectra are shown in Fig. 5a,b.

During laser irradiation, the temperature of the pigment suspensions increased slightly, and the pigments might be cleaved by the thermal energy of the suspension to a certain extent. To check the possible effects of elevated temperatures, the pigments were heated up to 200°C in a separate study. The suspensions were investigated by chromatography after being heated up to 50°C, 100°C, 150°C or 200°C without laser irradiation. However, the chromatogram of the heated suspensions remained nearly unchanged (data not shown).

DISCUSSION

Many of tattooed people decide to remove their tattoos. Besides adverse reactions (5,6,16,19,20) of the tattoo pigments itself, the main reasons for removing tattoos are improved self-image or social stigmatization. Traditional modalities are the removal of the pigment-containing skin using salabrasion (21), cryosurgery (22), surgical excision (23) or CO_2 laser application (24). However, these methods induce permanent scarring. With selective photothermolysis (13), tattoo removal is associated with significantly lower risk of scarring (25).

Therefore, the removal of tattoos by laser irradiation is a widespread therapy used by physicians of different fields. Tattoos were treated using different laser systems such as ruby lasers (694 nm), alexandrite lasers (755 nm) or Nd:YAG lasers (532, 1064 nm) at the respective wavelengths (26–28).

The laser wavelength of 532 nm was used in view of the absorption spectrum determined previously (3). In addition, at this wavelength the chromatograms show clear absorption for the

Table 1. The table shows the amounts of decomposition products before and after laser irradiation regarding the pigments CR or I8. The products found are 2,5-DCA, 1,4-DCB, 2-MNA and 4-NT

Decomposition product	CR before irradiation	CR after irradiation	CR before irradiation	CR after irradiation
µg/mL				
2-MNA	1.6 ± 0.3	53.1 ± 10.1		
4-NT	1.0 ± 0.2	44.7 ± 8.2		
2,5-DCA			11.8 ± 0.3	79,6 ± 1,4
1,4-DCB			<0.5	32.6 ± 0.4

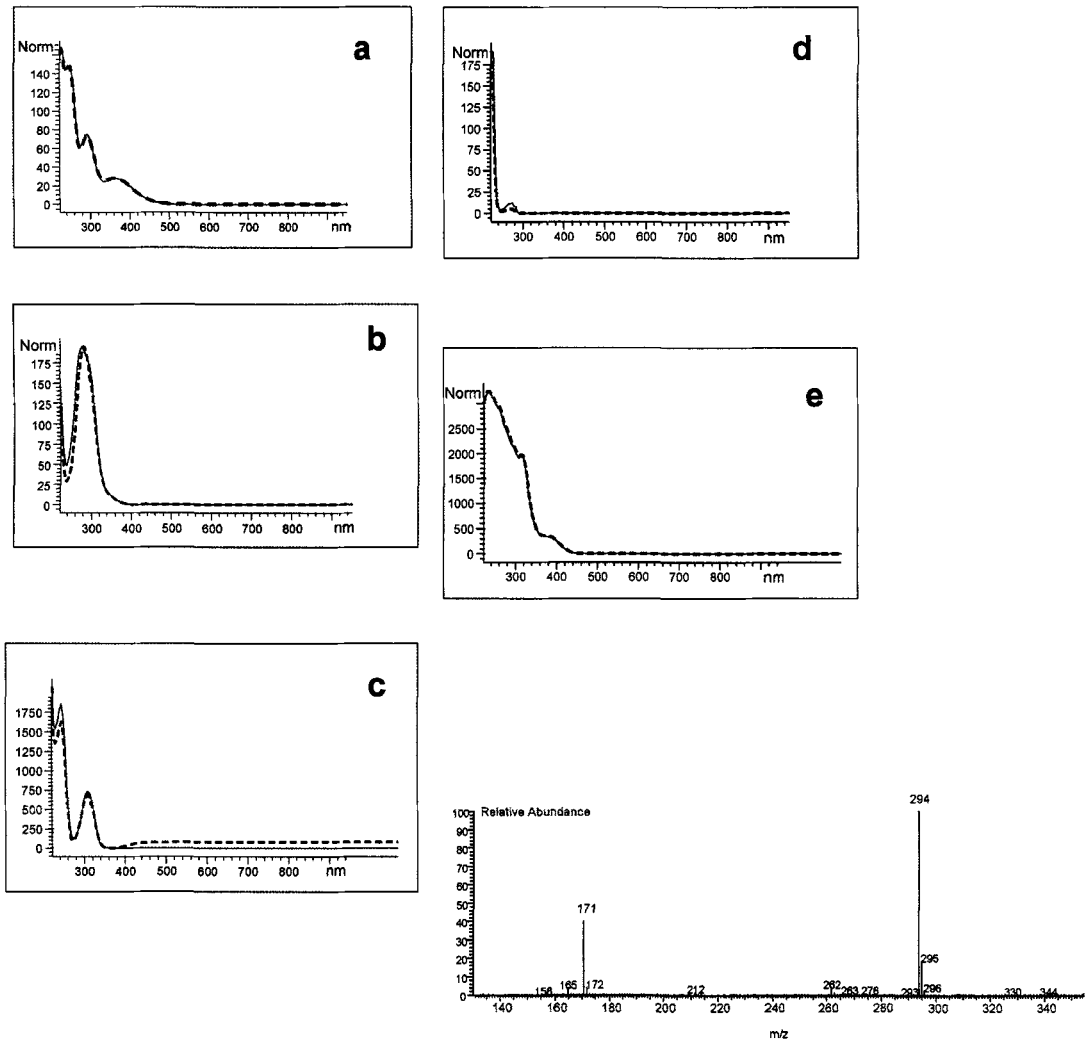


Figure 4. (a–e) The panel show the UV–VIS spectra of the decomposition products and the standards. Norm is the maximum normalization of the full wavelength range of the spectra shown. The solid lines account for the standard substances and the dashed lines for the decomposition products. a: Spectra of the peak at 21.6 min in Fig. 2b and the 2-MNA standard. b: Spectra of the peak at 31.7 min in Fig. 2b and the 4-NT standard. c: Spectra of the peak at 34.0 min in Fig. 3b and the 2,5-DCA standard. d: Spectra of the peak at 42.5 min in Fig. 3b and the 1,4-DCB standard. e: Spectra of the peak at 44.6 min in Fig. 2b and the naphthol-AS standard. f: Mass spectrum of methoxy-naphthol-AS (M = 293 g/mol) after liquid chromatography–mass spectroscopy coupling. The spectrum shows the positive Electrospray Ionization mass 294 and the mass 171 after separation of 1-methoxyaniline (123).

pigments but not for the contaminating compounds naphthol-AS or methoxy-naphthol-AS (Figs. 2a, 3a, and 5a,b). Regarding 694 or 1064 nm, the pigments used show an absorption coefficient close to zero. However, a substantial absorption of light energy in the pigments is necessary. In that case the corresponding energy is converted predominantly to heat, leading to a substantial increase of the temperature of the molecule and consequently inside the pigment particle. That leads to both the demolition of the pigment crystals and the chemical change of pigment molecules. It appears reasonable that both effects contribute clinically to the fading of the tattoo color after laser treatment. In case of a suboptimal wavelength, hardly any or no fading of the tattoo color should take place.

Despite the numerous patients treated with lasers by physicians, there are no investigations of the decomposition products of the tattoo pigments. When applying the laser energy to the pigment suspension, the results show cleavage of the tattoo pigments and a significant increase (up to 45-fold) of decomposition products. The products are 2-MNA, 4-NT, 2,5-DCA, 1,4-DCB, and naphthol-AS or 1-amino-naphthol-AS. Therefore, the fluence used for the study is within the range of clinical settings of 2–4 J/cm^2 (29–31).

4-NT is toxic as shown with human lymphocytes (32). 5-Nitro-*o*-toluidine, which is also designated to 2-MNA, may cause liver dysfunction as shown with workers from a hair dye factory (33). In addition, 2-MNA is a carcinogenic substance as shown by Sayama *et al.* (34) using *Salmonella typhimurium* YG, similar to other dinitro-toluenes. 1,4-DCB has been reported to cause tumors in the kidney of male rats and in the liver of male and female mice (35), whereas 2,5-DCA was capable of inducing nephrotoxicity in rats (36). Naphthol-AS or 1-amino-naphthol-AS leads to skin irritation. The toxicology of these compounds have not been completely investigated so far.

HPLC before laser irradiation (Figs. 2a and 3a) shows that the tattoo colorants are already contaminated with a variety of other compounds, among them are the same compounds produced by laser irradiation. These impurities are possibly caused by the chemical synthesis of the colorants. One has to take into account that these colorants have never been produced for application in

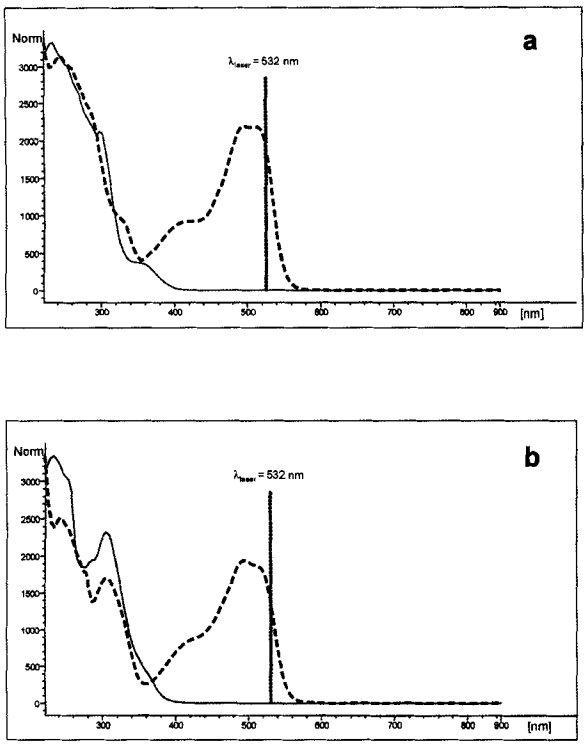


Figure 5. a: The absorption of naphthol-AS (solid line) in the HPLC at 44.6 min and P.R.22 (CR) (dashed line) at 56.1 min. b: The absorption of methoxy-naphthol as (solid line) in the HPLC at 43.7 min and P.R.9 (I8) (dashed line) at 60.9 min. The wavelength of the laser (532 nm) is added for comparison. Norm is the maximum normalization of the full wavelength range of the spectra shown.

humans, although they are injected into the skin, like medical drugs. The chemical industry produces such colorants to stain consumer goods. Therefore, they have not taken care of these impurities detected by HPLC in this investigation.

Moreover, laser irradiation induced many other products as shown by chromatography (Figs. 2b and 3b). These products remained unidentified so far because of the complexity of chemical reactions induced by the laser light. As reported recently, all these compounds may cause adverse effects in the skin with an extensive urticarial and indurated reaction 30 min after laser treatment of a tattoo (37). It is well known that tattoo pigments are transported through blood vessels or the lymphatic system in the human body, *e.g.* to the lymph nodes (38) or even to other organs such as the liver. Similarly, the laser-induced decomposition products could be transported in the body. Unfortunately, there are no investigations regarding the transport of the tattoo pigments and their impurities after tattooing as well as for the decomposition products after laser treatment.

CONCLUSIONS

So far, the laser treatment of tattoos cannot be considered as dangerous. However, because of the high number of patients undergoing laser treatment of tattoos, it is high time to assess the risk of the tattoo laser treatment in humans. The first results of this analytical procedure shows that the chemical identity of decomposition products and their respective amounts can be determined very accurately, at least in these *in vitro* experiments. The analysis of the decomposition products has been now established in a HPLC solvent. However, because of the location of tattoo pigments *in vivo*, an aqueous environment must be considered when performing a risk assessment. To perform that risk assessment of laser-induced decomposition products in humans, the concentration of a tattoo colorant in the skin must be determined. Unfortunately, that concentration is unknown. Thus, it is an important and upcoming goal to determine the amount of pigment inside the skin, which interacts with the laser at a fluence used in laser therapy.

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