Consumer Protection

Tattoo ink: A Closer Look

Tattoo removal using laser radiation can carry health risks depending on the breakdown products formed. Scientists from the Federal German Institute for Risk Assessment and Consumer Safety have now shown that pyrolysis GC/MS can be used to simulate the breakdown process and determine the compounds formed from a given ink during laser treatment. Phthalocyanine blue (B15:3), for example, was shown to form a cell poison in the process. Clear as ink? It is hoped that pyrolysis GC/MS can help consumer safety agencies determine whether certain tattoo inks should be approved or banned, in light of possible laser removal treatment.

By Guido Deussing

There can be very good reasons to have a tattoo removed: An allergic reaction to the pigments used; a different motif may be required, commensurate with age and experience; the name embedded in your skin may no longer be the love of your life; older images may have faded or not look as good as they once did due to the aging canvas; or the tattoo may stand in the way of your next career move. In the past, having such a permanent fixture removed required the use of a scalpel or etching or sanding of the skin - an unpleasant process. Fortunately for those suffering from tattoo remorse, techniques have recently become available to remove tattoos relatively gently without leaving visible scars or traces.

Laser treatment is preferred but it is not without risk

It may be a gentler treatment than previous generations of tattooees had to endure, but using a laser could entail certain health risks. At least that is what a joint study by the Federal Institute for Risk Assessment and Consumer Safety and the Laser Department of the Elisabeth Hospital points to. Both are located in Berlin, Germany. The study was published in "Scientific Reports"[1]. In it, Ines Schreiver, Christoph Hutzler, Peter Laux, Hans-Peter Berlien and Andreas Luch report that toxic and even carcinogenic compounds are formed during laser treatment of the copper-containing tattoo pigment phthalocyanine blue (B 15:3). The scientists simulated the fragmentation process using pyrolysis GC/MS and compared the fragments formed with laser breakdown products determined by Dynamic Headspace (DHS) in a separate experiment involving two-dimensional GC coupled with Time-of-Flight Mass Spectrometry (TOF-MS). Among the compounds determined were: 1,2-Benzenedicarbonitrile (BDCN), benzonitrile (BCN), 2-butanone, benzene, and hydrogen cyanide as the main fragmentation products.

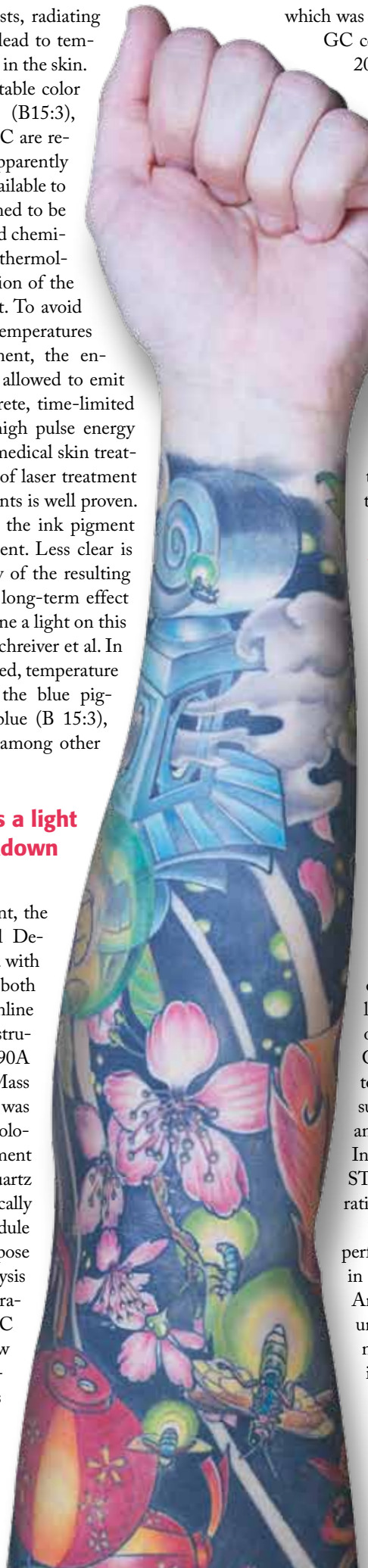
Laser radiation meets tattoo pigments

In clinical dermatology, ruby lasers are regularly used to treat pigmented spots, i.e. liver spots and to remove tat-

toos. According to the scientists, radiating the skin with a ruby laser can lead to temperatures of more than 1000 °C in the skin. To break down the relatively stable color pigment phthalocyanine blue (B15:3), temperatures in excess of 800 °C are required. Bleaching of what is apparently the only blue tattoo pigment available to dermal needle workers is assumed to be the result of a thermally induced chemical breakdown process (photo-thermolysis) equivalent to an atomization of the pigment, Schreiver et al. report. To avoid damaging the skin at the high temperatures generated during laser treatment, the energy rich laser light cannot be allowed to emit continuously, but only in discrete, time-limited pulses. The ruby laser has a high pulse energy level making it well suited for medical skin treatment purposes. The efficiency of laser treatment in breaking down tattoo pigments is well proven. It can be observed directly by the ink pigment bleaching effect during treatment. Less clear is the exact identity and quantity of the resulting chemical derivatives and their long-term effect on the human organism. To shine a light on this matter was the stated goal of Schreiver et al. In order to imitate the laser induced, temperature dependent decomposition of the blue pigment copper phthalocyanine blue (B 15:3), pyrolysis - GC/MS was used among other techniques.

Pyrolysis GC/MS shines a light on laser induced breakdown fragments

To pyrolyze the B 15:3 pigment, the scientists relied on a Thermal Desorption Unit (TDU), equipped with a pyrolysis module (PYRO), both from GERSTEL. For the online coupled GC/MS analysis, an instrument setup consisting of a 7890A GC and a 5975C inert XL Mass Selective Detector (MSD) was used, both from Agilent Technologies, Inc., Palo Alto. The pigment sample was placed inside a quartz liner, which was automatically transferred to the PYRO module by the GERSTEL MultiPurpose Sampler (MPS). The pyrolysis step took place within a temperature range from 500 to 1,000 °C lasting 6 sec. A carrier gas flow of 1 mL/min (Helium) transported the pyrolysis fragments via the Cooled Injection System (CIS) PTV type GC inlet, which was kept at a temperature of 260 °C, to the GC column (HP-Plot/Q, 30 m, 0,32 mm x 20 µm ID from Agilent Technologies). Analyte separation was performed using a temperature gradient: Initial temperature 50 °C (2 min); 10 °C/min to 260 °C (10 min); EI ionization; full scan mode detection 10 to 550 m/z. Pyrolysis fragments were determined using the NIST MS library (US-NIST, National Institute of Standards and Technology, 2011 MS Library). Fragments found were: 1,2-Benzenedicarbonitrile (BDCN), benzonitrile (BCN), benzene and Hydrocyanic acid (HCN). Schreiver et al. reported that with increasing pyrolysis temperature the amount of fragmentation products formed also increased.



Accuracy of the pyrolysis simulation verified

In order to assess whether pyrolysis of pigment B 15:3 produced the same fragments as laser radiation breakdown, the scientists produced different water based dispersions of the pigment and subsequently irradiated these with a pulsed ruby laser. The irradiated samples were then analyzed, first by Dynamic Headspace (DHS)-GC/MS to quantify the volatile compounds HCN and benzene. The previously described MPS-DHS-TDU-GC/MS system was used. D6-benzene was used as internal standard. Secondly, ethyl acetate extracts of the irradiated dispersions were analyzed by two-dimensional GC/Time-of-Flight-MS (Leco-Pegasus 4D GCxGC-ToF-MS) specifically in order to quantify BDCN and BCN. This was successfully achieved using benzyl nitrile and Benzyl alcohol as internal standards. In all cases, Schreiver et al. used the GERSTEL MPS for automated sample preparation and introduction.

The DHS-GC/MS determination was performed by thermostating the samples in the agitator for three minutes at 30 °C. Analytes were purged with a 100 mL volume of nitrogen (N_2) at a flow rate of 50 mL/min and focused in a sorbent trap inside a TDU tube (Carbopack B+X/Carboxen 1000). Analytes were desorbed inside the TDU and re-focused in the CIS at -50 °C. After 12 sec, the

CIS was heated to 40 °C (5.5 min) and then heated within seconds to 240 °C (5 min). The TDU and transfer system temperature were then kept constant at 260 °C for the remainder of the run. Sample introduction to the GC column was performed in splitless mode. The GC oven initial temperature was set to 40 °C (0.5 min) and then ramped at 10 °C/min to 260 °C (10 min). The mass range from 10 to 350 m/z was scanned with parallel Single Ion Monitoring (SIM) of the masses 27, 28, 78, and 84 m/z, each with a dwell time of 40 ms.

The GCxGC-TOF-MS determination of fragments generated during laser treatment was approached as follows: To a 196 µL sample placed in a 2 mL vial, the MPS added an internal standard and subsequently performed a liquid/liquid extraction with ethyl acetate for one hour in the agitator. A 1.5 µL aliquot of the resulting extract was introduced to the GC inlet for two-dimensional separation: The first dimension was based on a Restek Rxi-5Sil MS column (20 m, 0.25 mm, 0.25 µm ID), the second on a Restek Rxi-17Sil MS (1 m, 0.18 mm, 0.18 µm ID) column. The initial oven temperature was set to 70 °C (1 min), followed by a 15 °C/min ramp to 120 °C (0 min), 8 °C/min to 150 °C (0 min) and finally 25 °C/min to 330 °C (4 min). Effluent fractions from the first column were trapped and subsequently released into the second column using thermal modulation based on a cryofocusing temperature of -80 °C. For the second dimension separation, the temperature program was set a few degrees higher than the first dimension program. The ion source temperature was set to 250 °C, the transfer line to the MS to 295 °C. Mass spectra were recorded at a rate of 200 Hz scanning from 35 to 500 m/z. BCN and BDCN were unequivocally identified and quantified.

Assessing toxicity

For Ines Schreiver and her colleagues, a key question in this matter is whether laser induced breakdown of phthalocyanine blue actually generates degradation products in amounts that are unsafe – apart from the fact that hydrocyanic acid and benzene are known to be acutely toxic and a carcinogen, respectively. To find an answer, the scientists used a special experimental design based on human cells, which were exposed to sodium cyanide (NaCN) solutions of different concentrations. DHS-GC/MS was subsequently used to determine the amount of HCN liberated by the cells in order to get a picture of the kinetics of the HCN formation and its distribution in adjacent tissue following exposure to laser radiation. The scientists quote the U.S. Centers for Disease Controls and Prevention (CDC) as source for the fact that the lethal dose of HCN is around 2 mg/kg body weight "in most animal species" and that the "immediately dangerous to life or health concentration (IDLH) in air is 50 ppm (www.cdc.gov/niosh/idlh/74908.html). According to Schreiver and her colleagues, investigations with laser treatment of the tattoo pigment phthalocyanine blue have shown that toxicologically relevant concentrations of HCN are formed and that these have a significant effect on the ability of the cell to survive. In their totality, the experiments indicate that during laser treatment of light resistant phthalocyanine blue B 15:3 in skin, toxic fragments are formed in amounts that could most probably influence both the skin locally and systemically other tissue in the organism. Further studies will be needed using human skin ex vivo to investigate the formation of HCN and benzene during laser treatment as well as the consequences of their presence. Further, the scientists suggest that all this information be taken into account and in future quality assessment and approval processes for tattoo inks.

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Literature

[1] Ines Schreiver, Christoph Hutzler, Peter Laux, Hans-Peter Berlien & Andreas Luch, Formation of highly toxic hydrogen cyanide upon ruby laser irradiation of the tattoo pigment phthalocyanine blue; Scientific Reports 5, Article number: 12915 (2015) (www.nature.com/articles/srep12915 , 2017/02/15)

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GC/MS system similar to the one used at BfR in Berlin: GERSTEL MPS with TDU 2, PYRO, and DHS mounted on a GC/MS system from Agilent® Technologies.