Journal of Inorganic and General Chemistry
ZAAC
Zeitschrift für anorganische und allgemeine Chemie
www.zaac.wiley-vch.de
RESEARCH ARTICLE

DOI: 10.1002/zaac.202300213

Special Issue

Mass spectrometric analysis of transition metal complexes formed through contact of artificial sweat with circulating Euro coins

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Dedicated to Professor Rhett Kempe on the occasion of his 60th birthday.

It is well-known that some metals/alloys sensitize the skin of susceptible individuals. Trace amounts of metals and chemical compounds containing metal ions can affect human health, i.e., cause allergy or inflammation. However, the details of this metal-induced allergic reaction are not well understood. Complexes of nickel, copper, and zinc are known to be easily formed through contact of metal alloys with ubiquitously available organic compounds such as triacylglycerols, amino acids, urea, and fatty acids found in skin secretions or skin care products. Thus, an analytical technique is required to characterize and identify metal complexes formed on metallic everyday products, ideally offering a chemically specific, laterally resolved view of allergy-causing processes. Here we present a mass-spectrometry-based analytical workflow to characterize metal-containing compounds formed on the surface of 1-euro coins when in contact with artificial sweat. This study identified different metal complexes by using tandem mass spectrometry (MS/MS). Surface imaging of 1-Euro coins employing laser-based mass spectrometry revealed the lateral distribution of Zn, Cu, and Ni complexes with low-micrometer resolution.

Introduction

Nickel ions with physiologically relevant ligands in small concentrations are spectroscopically difficult to detect. Therefore, nickel was not considered an element of biological importance for long.[1] However, the evidence of the essentiality of this element for plants goes back to the '70s of the twentieth century, when a group of researchers suggested the possible role of Ni in the metabolism of nitrogen through its participation in the structure of the enzyme urease.[2] In the 1980s, Eskew et al.,[3] demonstrated the essential role of Ni in the nitrogen metabolism of leguminous plants (soybeans), a role that was independent of the form of available nitrogen (NO_3^- or NH_4^+). The evidence generated by this research suggested the essentiality of Ni for higher plants. Nickel ions are required for the active synthesis and metabolism of urease in plant cells such as jack beans (*Canavalia sp.*), soybeans (*Glycine max*), rice (*Oryza sativa*), and tobacco (*Nicotiana tabacum*).[4]

Since then, nickel chemistry in biological systems has been intensively investigated, and several nickel-dependent enzymes have been discovered. Nickel is now considered an essential element in the active site of enzymes of animals, microorganisms, and plants.[5] Furthermore, it is suggested to be an essential micronutrient for the proper function of the human body.[6] However, nickel is also a toxin and it is thus important to better understand why cells select nickel ions among a pool of different and more readily available metal ions.

Gastritis can often be caused by the bacterium *Helicobacter pylori*,[7] which a urease test can detect. Nickel-containing urease contains two nickel(II) ions in the active site, forms carbon dioxide and ammonia (which protects *Helicobacter* against the stomach's hydrochloric acid), and a color change of an indicator simply detects ammonia.

Besides its biological occurrence, nickel furthermore is released into the environment by anthropogenic sources such as the combustion of fossil fuels (coal-fired power plants or the combustion of fossil oil)[8] and from mining as well as from agriculture (especially fertilizers).[9] People can develop allergies to some metals; nickel is one of the most common causes of allergic contact dermatitis.[10] Exposure typically occurs from

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Supporting information for this article is available on the WWW under https://doi.org/10.1002/zaac.202300213

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This article is part of a Special Collection dedicated to Professor Rhett Kempe on the occasion of his 60th birthday. Please see our homepage for more articles in the collection.

metallic items such as household products, jewellery, or coins.[11] The reaction of a nickel-allergic person, after contact with nickel items, is a local skin rush (Figure 1), that usually disappears after a few days.

However, much more severe effects can develop.[12] Molecular mechanisms of nickel allergy have been investigated.[13] Tests for a nickel allergy are usually done by exposing the individual to a small amount of a diluted nickel sulfate solution (tape stripping).[14] The sensitization and elicitation thresholds for nickel allergy and dermatitis differ at least 250 – fold, making it impossible to define a safe threshold level.[11a] There is a significant variation in the degree of sensitivity to nickel between individuals. Even tiny amounts of nickel can cause a reaction in nickel-allergic individuals.

Nickel allergy and dermatitis are sought to be prevented by limiting nickel exposure from items used for prolonged contacts. Nickel release is measured by a test technique called EN 1811 in which the test object is placed in an artificial sweat solution for one week, after which it is measured by atomic absorption spectroscopy or another suitable method.[10] According to the 'Nickel Directive', jewellery and other products intended to come into direct and prolonged contact with the skin shall not release too much nickel. In the case of post assemblies inserted into pierced ears and other pierced parts of the body, this should be less than 0.2 μg/cm^2/week and 0.5 μg/cm^2/week for other products destined for direct and prolonged contact.

While the first coins were made of precious metals such as gold and silver, less expensive metals such as copper and nickel were used soon after. For example, the 5-cent coin in the US, called a nickel, consists today of 75% copper and 25% nickel. This alloy is widely used for coins due to its silver color, corrosion resistance, resilience, and durability.[15] The compositions of coins often changed over time. Currently, nickel is widely used in the coins of the European Union. A total of two alloys are included in the metal composition of coins in circulation in the EU countries as of 2012. The main characteristics of the 1 Euro coin are a diameter of 23.25 mm, a thickness of 2.33 mm, and a mass of 7.5 g.[16] It contains a metallic outer ring that appears yellow (nickel brass), consisting of 75% copper, 20% zinc, and 5% nickel (Figure 2).

The white alloy in the center (cupro nickel) is 75% copper with 25% nickel. The composition of 1-euro coins can vary slightly, depending on the country that issues the coin. The skin contact of both alloys could, thus, potentially cause an allergic reaction in people sensitized by nickel. An increase in contact dermatitis has raised concerns because of the introduction of the new Euro coins in 2002.[17]

It is well known that to cause an allergic reaction, enough of the allergenic metal must be dissolved to enter the body through the skin. Solubilization of the allergenic metal (forming ions) can occur due to corrosion of the metal (or alloy) by itself or by body fluids. The corrosion rate, duration, and frequency of skin contact, nature, and concentration of the dissolved allergenic metal ions, play an important role in determining the extent of an allergic reaction.[15]

Several factors affect nickel release from coins, including the composition of the coins, their size, the solvents, and the immersion duration and temperature. A simple spot test for nickel release from surfaces is applying a solution of dimethylglyoxime (DMG), which gives a positive test due to the characteristic color of the formed nickel complex (Figure 3), well known in basic analytical chemistry protocols, where it can be used to quantify nickel gravimetrically. The test can also be used after the skin has been exposed to metal.[18]

In a quantitative nickel-release test (EN 1811), 1- and 2-Euro coins released approximately a mean of 86 μg/cm^2/week and a

Figure 2. 1-euro coins from a) France 1992, b) Germany 2002 and Spain 2007.

Figure 1. Skin rush of a nickel-allergic person in our lab after wearing a lab coat with buttons attached to nickel-containing metal rings.

Figure 3. Formation of the nickel dimethylglyoximate complex on a cotton swap.

mean of 99 µg/cm^2/week, respectively.[19] Therefore, the nickel release from the present 1- and 2-Euro coins exceeds the acceptable threshold for prolonged contact with human skin, which is considered to be potentially harmful to individuals with nickel allergies or sensitivities. Hence, coin handling is probably contributing to nickel dermatitis. To determine how much nickel and other metals have been deposited on the skin, two sampling methods were developed: finger immersion and acid wipe sampling. The amount of nickel present on the fingers of players, cashiers, sales assistants, caterers, and office staff was measured. Studies have revealed that some people, such as players, cashiers, sales assistants, caterers, and office staff, may have elevated levels of nickel on their fingers that surpass 0.035 grams per cm^{-2}. This can cause allergic reactions in 22% of those who are allergic to nickel.[20] For studies of skin exposure to nickel under standardized experimental conditions in the workplace and in the general setting, the acid wipe sampling technique is suitable.[19]

The EU is recommending for the testing of nickel from coins to use either an inductively-coupled plasma optical emission spectrometer (ICP-OES), or ICP mass spectrometer (ICP-MS), or an electrothermal excitation atomic absorption spectrometer (GFAAS). Regarding the importance of the uptake of the nickel ions from the surface of the coins, we investigated nickel compounds and complexes that form in contact of 1-Euro coins with sweat by using the molecularly-sensitive laser desorption ionization mass spectrometry (LDI-MS).

Results and Discussion

For our study, we used three circulating 1-Euro coins from Spain, Germany, and France that were cleaned and treated with artificial sweat. These coins were chosen for initial experiments to evaluate if AP-LDI MS (atmospheric pressure laser desorption/ionization mass spectrometry) is suitable for detecting molecular and elemental components from the coin surfaces shown in Figure 2.

Positively singly-charged nickel, copper, and zinc ions were detected, as shown in Figure S1. MS imaging of the 3D surface of a 1-Euro coin showed that Zn was most abundant in the outer ring, Cu was present in both inner and outer rings, and Ni was found primarily in the inner ring and that several metal-organic compounds were readily detectable from the surface. An exemplary AP-LDI MS imaging result of a cleaned 1-Euro coin is shown in Figure 4. The signals at *m/z* 207.8941 ($[C_4HO_3NNi+K]^+$), and *m/z* 116.9527 ($[CH_4NZn+Na]^+$) are shown in red, and green, respectively. The signals were assigned based on their accurate masses and isotopologue patterns using the XCalibur software (Figures S9).

Pixel coverages, computed for three 1-Euro coins as percentage of pixels with signal intensities of selected ions above a preset threshold value, are shown in Figure 5. As expected and consistent with reported coin compositions, Cu^+ ions had the highest pixel coverage, followed by zinc and nickel in the three coins.

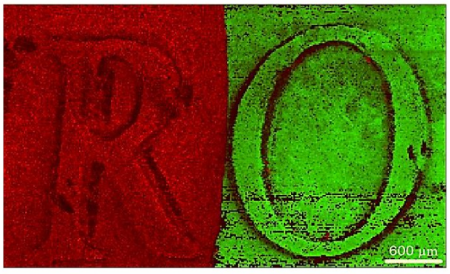

Figure 4. 3D-surface red-green overlay MS image of *m/z* 207.8941 (red, $[C_4HO_3NNi+K]^+$), and *m/z* 116.9527 (green, $[CH_4NZn+Na]^+$). MS images of the two signals were generated from a cleaned 1-Euro coin (2002, Germany) with 320×189 pixels; 15 µm pixel size; *m/z* bin width: $\Delta(m/z)/(m/z) = \pm 5$ ppm.

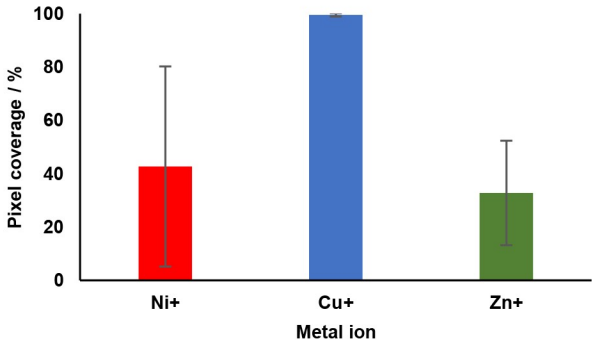

Figure 5. Average pixel coverages of Ni^+, Cu^+ and Zn^+ ions in scanned area (see Figure 4) of three 1-Euro coins from Spain, Germany and France.

A comparison of the three coins revealed that the pixel coverage of Ni^+ in the 1-Euro coin from France (1999) was higher than in the 1-Euro coins from Spain (2007) and Germany (2002) (Figure 6). We believe that the higher Ni^+ pixel coverage on the 1-Euro coin from France is due to the fact that the French coin was produced in 1999 and the nickel directive

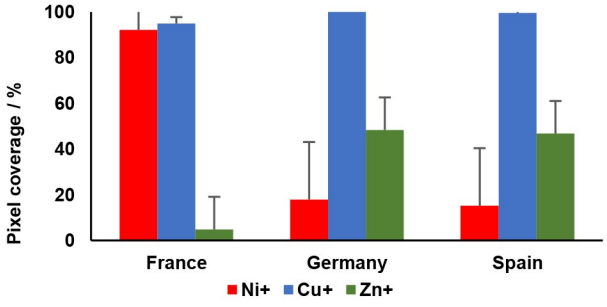

Figure 6. Pixel coverage percent of Ni^+, Cu^+, and Zn^+ ions in scanned area of 1 Euro coin France 1999, Germany 2002 and Spain 2007.

guidelines, which were adopted in 1994, took effect in 2000 and have been implemented since 2001.

Even if pixel coverage is not a quantitative measure for bulk concentration, these results demonstrate that AP-LDI MS is capable to spatially probe metal appearances in coins and that these results are in line with reported region- and time-specific production processes.

Besides metal ions, metal-organic ions from the coin surface were investigated. Examples besides those of Figure 4 are *m/z* 178.8832, assigned to $[CH_4NCu_2+Na]^+$ and *m/z* 258.8152 assigned to $[CH_5ONCu_3+Na]^+$, shown in Figure 7. The signals were assigned based on their accurate masses and isotopologue patterns using the XCalibur software (for isotopologue patterns, MS images and tandem mass spectrum see Figures S2, S3, S4, S5).

In addition, transition metal complexes formed during skin contact with Euro coins were studied. Since sweat can play an important role in enhancing metal allergies, corrosion products produced by sweat on the surface of a 1-Euro coin were studied. In this study, three standard solutions, including urea (0.1 %), lactic acid (0.1 %), and artificial sweat solution (ASS), were sprayed homogeneously onto the surface of a cleaned 1-Euro coin. The color of one of the coins after spraying the urea solution changed to green in some parts after a day, as shown in Figure 8a, visibly indicating that metal ions had reacted with the urea solution. AP-LDI MSI experiments probed the corrosion products on the surface and detected a signal at *m/z* 140.9720, assigned as $[CuCH_4N_2O+H_2O]^+$ (MS image before and after spraying ASS, see Figure 9; experimental and theoretical isotopologue patterns of $CuCH_4N_2O+H_2O]^+$, see Figure S8). Although the lactic acid solution did not cause any apparent change in the coin surface (Figure 8b), an unknown new signal at *m/z* 158.0821 was detected.

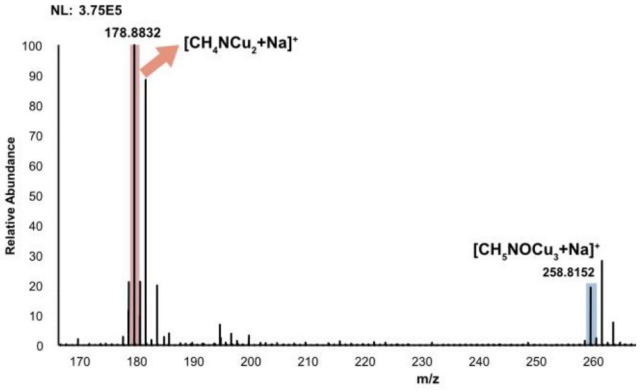

Figure 7. Positive-ion mode AP-LDI mass spectrum of *m/z* 178.8832 that was assigned to $[CH_4NCu_2+Na]^+$ and *m/z* 258.8152 assigned to $[CH_5ONCu_3+Na]^+$. Spectra were generated from a cleaned 1-Euro coin (2002, Germany) by averaging from 320×189 pixels; 15 µm pixel size. For isotopologue patterns see Figure S2.

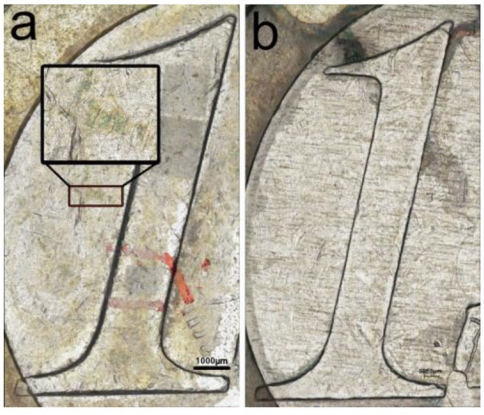

Figure 8. On a 1 Euro coin. a) urea 0.1 % solution, b) lactic acid 0.1 % solution after one day. The colour change is shown in a black rectangle, and the red marker shows the MALDI Mass Spectrometry analysis region.

To confirm the identity of $[Cu(CH_4N_2O)+H_2O])^+$, formed on the surface of 1-Euro coins after spraying ASS, we compared tandem mass spectra from the surface (Figure S6) with those from the desired metal complex generated from a standard solution of 1 mol/L copper(II) chloride dihydrate ($CuCl_2 \cdot 2\ H_2O$) and 0.1 % (m/m) urea (Figure S7). α-cyano-4-hydroxycinnamic acid (CHCA, purity 97 %, Sigma-Aldrich, USA) was used as a matrix for tandem mass spectra of the standard. Due to the low signal intensity of the complex measured by AP-LDI from the surface, the isotopologue patterns from tandem measurements from the coin surface and from the standard are only partly identical, but still confirm the identity of the compound.

Compared to cleaned Euro coins, the relative abundance of Ni^+ and Cu^+ ions increased on the surface of coins sprayed with solutions of urea (1 g/L) and lactic acid (1 g/L). Comparing the relative abundances of metal ions on the surface showed that after three days, the lactic acid solution produced a higher concentration of Ni^+ and Cu^+ ions compared to the urea solution, while after six days, the urea solution yielded a higher concentration of Ni^+ and Cu^+ ions compared to the lactic acid solution. The results provide an explanation for how lactic acid and urea impact the production of metal ions (by either oxidation of metals or by release from already oxidated metal compounds such as metal oxides or metal sulfides), particularly Cu^+ ions. As nickel allergy follows contact with the allergen, it can take 24 to 72 hours before symptoms manifest. These data suggest that acidic sweat can increase Ni^+ content in the first

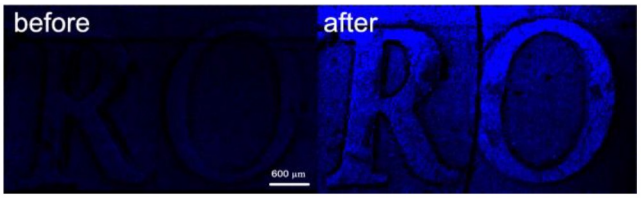

Figure 9. AP-LDI MS image of *m/z* 140.9728 (blue, $[Cu(CH_4N_2O)+H_2O])^+$) before and after spraying with ASS. MS images were generated with 325×193 pixels; 15 µm pixel size; *m/z* bin width: $\Delta(m/z)/(m/z) = \pm 5$ ppm.

three days, resulting in an aggravated allergy, shown in Figure 10. A comparison of the pixel coverages of the three metal ions on the scanned area before and after spraying ASS, is shown in Figure 11.

Comparison of 3D-surface RGB MS images of the scanned area before and after spraying ASS also indicate the increase of pixel coverage of Ni^+ ions (Figure 12).

As shown by AP-LDI mass spectrometry, sweat increases the production of metal ions, especially nickel metal ions, which may play an important role in nickel allergy development. The increase of nickel metal ion abundances after one week is more influenced by urea, one of the main components of sweat, than by lactic acid.

Conclusions

A metal object interacting with sweaty skin will cause sensitive skin to become itchy and prickly within 15 to 20 minutes. Wearing these metallic items for several hours without a problem is common if people don't sweat.[21] So far, there is no clear understanding of how metals induce allergic reactions.

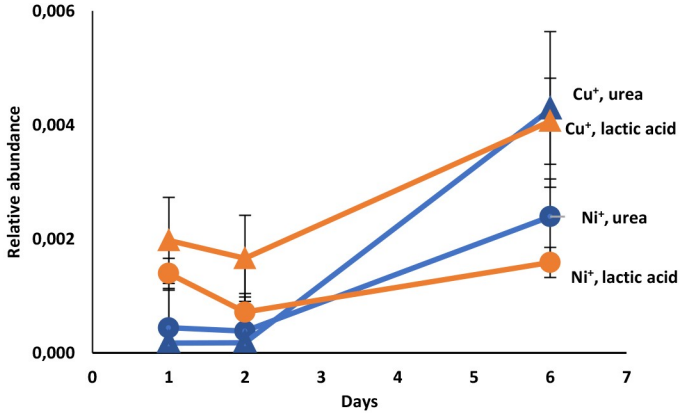

Figure 10. Relative abundance changes of Ni^+ and Cu^+ ions detected after spraying coin surfaces with urea and lactic acid 0.1% solutions during 6 days; signals normalized to TIC.

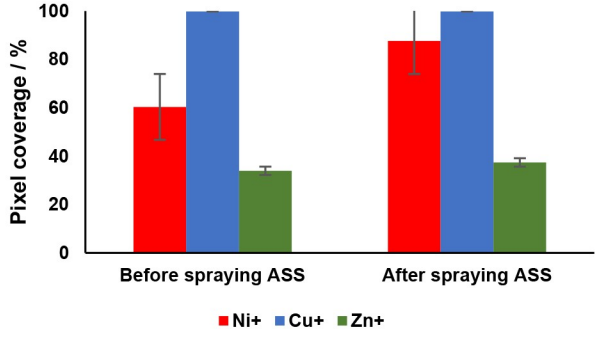

Figure 11. Pixel coverages of Ni^+, Cu^+ and Zn^+ ions in scanned area before and after spraying ASS for 6 days.

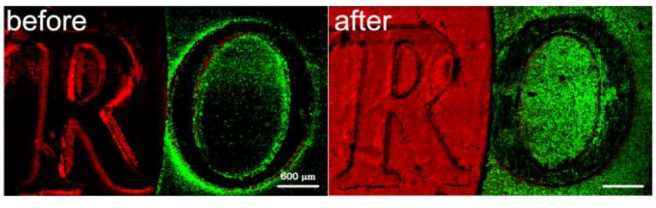

Figure 12. 3D-surface RGB MS images of *m/z* 57.93480 (red, $[Ni]^+$) and *m/z* 63.92860 (green, $[Zn]^+$), a) before and b) after spraying with ASS; *m/z* bin width: $\Delta(m/z)/(m/z) = \pm 5$ ppm

Metal alloys such as nickel, copper, and zinc are known to easily form complexes with organic compounds found in skin secretions, such as triacylglycerols, amino acids, urea, and fatty acids. Identifying metal complexes formed on metallic surfaces of everyday products requires an analytical technique that offers a chemically specific, laterally resolved view of the allergy-causing processes. The summed mass spectrum of the scanned area of the 1-euro coin revealed different metal complexes with high intensities. The signal at *m/z* 178.8832 was assigned to $[CH_4NCu_2 + Na]^+$ and the signal at *m/z* 258.8152 to $[CH_5ONCu_3 + Na]^+$ (Figures S3, S4). Depending on material and usage time, coins lose their original color, appearance, and pits over time. Sweat is the main cause of these surface alterations, gradually damaging the coin's surface. We studied the effects of lactic acid and urea, which are the main components of sweat, on the surface of a 1-euro coin to identify corrosion products that might cause skin allergies. Compared to urea solution, lactic acid solution produced more metal ions in the first three days. Furthermore, a new metal complex at *m/z* 140.9728 $[Cu(CH_4N_2O) + H_2O])^+$ was assigned in positive-ion mode. Limitations of our approach are variations of limits of detection of different metal-organic substances. Physiologically relevant compounds might be present on the surface of coins which are not detectable by our method. However, the 3D-surface red-green overlay MS image of *m/z* 207.8941 (red, $[C_4HO_3NNi + K]^+$)

and *m/z* 116.9527 (green, $[CH_4NZn+Na]^+$) in Figure 4 and the AP-LDI MS image of *m/z* 140.9728 (blue, $[Cu(CH_4N_2O)+H_2O])^+$) in Figure 9 shows that relevant Zn-, Cu- and Ni-containing compounds were detected with high signal intensities on the coin's surface. The method is able to investigate a large variety of other metal complex classes, provided that the signal intensity is high enough for tandem mass spectrometry. Still, we cannot completely exclude that some further compounds form that we did not detect.

Our findings do not allow us to solve the health issues caused by nickel allergy; however, having identified how the metals are dissolved from the coin surface, we will now start to investigate how the metal ions are penetrating the skin when causing the allergic reaction.

Experimental Section

Coins

For this study, coins were selected from the wide-ranging circulation.

Solutions and reagents

Sodium chloride (Sigma-Aldrich, Steinheim, Germany); lactic acid (Sigma-Aldrich); urea (Fluka™, urea ≥99.5%, Fisher scientific, Schwerte, Germany); 1 mol/L and 0,1 mol/L aqueous sodium hydroxide solution (Sigma-Aldrich). α-cyano-4-hydroxycinnamic acid (CHCA, purity 97%, Sigma-Aldrich) was used as a matrix in MALDI experiments. Matrix solution was prepared in a concentration of 10 g/L in acetonitrile/water 7:3 v:v with addition of 0.1 vol% trifluoroacetic acid (TFA, uvasol for spectroscopy, Merck, Darmstadt, Germany; acetonitrile uvasol, Merck; water HiPerSolv Chromanorm for HPLC, filtered at 0.2 µm, VWR, Darmstadt, Germany). Copper(II) chloride dihydrate ($CuCl_2 \cdot 2\ H_2O$) was purchased from Sigma-Aldrich.

Solutions for MS and MSI analysis

Lactic acid solution and urea solution were prepared in a concentration of 0.1% m/m in deionized water. An artificial sweat solution containing sodium chloride (0.5% m/m), lactic acid (0.1% m/m), and urea (0.1% m/m), dissolved in deionized water was prepared. The pH was adjusted to 6.5 by adding sodium hydroxide solution, following EN 1811, the reference method for testing in compliance with the EU Nickel Directive.

$[Cu(urea)]^+$ standard solution for MALDI MS measurements was prepared by mixing 1 mol/L copper(II) chloride dihydrate ($CuCl_2 \cdot 2\ H_2O$) and 0,1% (m/m) urea in 1:1 CH_3OH/H_2O. The solution were premixed in a ratio of 1:1 (v:v:) with CHCA matrix solution.

Sample preparation

Volumes of 200-µl of the lactic acid solution, the urea solution and the artificial sweat solution were sprayed onto pre-washed 1-Euro coins with a flow rate of 10 µl/min, an N_2 pressure of 1 bar and a rotation of 500 rpm using an ultrafine pneumatic sprayer system (SMALDIPrep, TransMIT GmbH, Giessen, Germany). The three coins were kept at a temperature of 37±2 °C for a week at normal laboratory atmosphere. An optical image of the coins was recorded with a Keyence VHX-5000 digital microscope (Keyence Deutschland GmbH, Neu-Isenburg, Germany) equipped with a VH–Z250R objective lens. Finally, the coins were inserted into the ion source of the mass spectrometer for 3D-surface AP-LDI MSI measurements.

Sample preparation of standards

One microliter of standard solution was deposited on a microscopy glass slide used as an MS target. After evaporation of the solvent, a 3D optical image of the sample was recorded by means of the digital microscope. Finally, the glass slide was inserted into the ion source of the mass spectrometer.

Instrumentation

Imaging experiments were performed on a Q Exactive HF orbital trapping mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with an autofocusing AP-SMALDI[5] AF ion source (TransMIT GmbH). Fifty UV-laser pulses per pixel at a frequency of 100 Hz were used to desorb and ionize sample atoms and/or molecules. Step sizes between 15 and 25 µm were chosen for imaging experiments according to the sample size (region of interest, ROI). The ion injection time was set to 500 ms, the s-lens level was set to 100 arbitrary units, and the capillary temperature was set to 250 °C. An acceleration voltage of 3.0 kV was used in the ion source. All measurements were performed in positive-ion mode with a mass resolution of 240,000 at *m/z* 200. The mass spectrometer was calibrated with known matrix signals prior to LDI or MALDI measurements, resulting in a mass accuracy of better than 2 ppm root mean square error (RMSE).

3D-surface AP-LDI imaging measurements

Autofocusing AP-LDI MSI experiments were performed using 25 µm step size with 177x120 pixels for 1-euro coin (2007, Spain), 25 µm step size with 320x189 pixels for 1-euro coin (2002, Germany) and 15 µm step size with 198x122 pixels for 1-euro coin (1999, France). The mass spectrometer was calibrated with known Cu^+ signals at *m/z* 62.92905 in RecalOffline software. Processing, evaluation and MS image generation were performed in Mirion software.

Data evaluation and image generation

Q Exactive Tune (version 2.4, Thermo Fisher Scientific, Bremen, Germany) was used for data acquisition. The SMALDIControl software (V1.1-118, TransMIT GmbH) was used for image acquisition and for system control and autofocusing 3D-surface operation.[22]

Mirion software package (v3.2.64.29) (TransMIT GmbH) was used for data evaluation and image generation. The absolute mass variance of spectra was set to 0.005 u.[23] The bin width was adjusted to 0.005 u. No TIC normalization was applied in the image creation process.

XCalibur (version 4.0.27.13, Thermo Fisher Scientific) was utilized to display mass spectra. Ion images of selected *m/z* values were generated using MIRION imaging software with a mass bin width of *m/z* ±5 ppm from the accurate mass. MS images were normalized to the highest intensity measured for each ion separately. No further image processing steps such as smoothing or TIC normalization were used. Red-green-blue (RGB) overlay images were obtained by selecting and overlaying *m/z* bins for the three channels.

Acknowledgements

Financial support by the German Academic Exchange Service – Graduate School Scholarship Programme (DAAD-GSSP) "Chemistry for Life" and the Giessen Graduate Center for the Life Sciences GGL is gratefully acknowledged (A. R.). Technical support by Thermo Fisher Scientific (Bremen) GmbH and by TransMIT GmbH is gratefully acknowledged. Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest

B.S. is a consultant of TransMIT GmbH, Giessen, Germany. The other authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: LDI mass spectrometry imaging · skin allergies · metal allergies · transition metal complexes

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Manuscript received: October 15, 2023
Revised manuscript received: December 22, 2023
Accepted manuscript online: December 28, 2023

