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In vitro studies of copper release from powder particles in synthetic biological media

Klara Midander*, Inger Odnevall Wallinder, Christofer Leygraf

Division of Corrosion Science, Department of Materials Science and Engineering, School of Industrial Engineering and Management, Royal Institute of Technology, Drottning Kristinas väg 51, 100 44 Stockholm, Sweden

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Copper release rates from particles are essential to assess potential health aspects.

Abstract

The aim of this paper is to provide quantitative data on copper release from powder particles of different copper materials, including artificial copper patina, Cu_2O and metallic Cu, when exposed to different synthetic biological media to simulate an inhalation scenario and/or skin contact. Generated data may contribute in risk assessment of potential health effects following exposure to and handling of various copper materials. All tests were performed in vitro to determine total copper concentrations, release rates of total copper, and to elucidate its time-dependence. The copper release process was interpreted in terms of specific surface area, surface morphology-, and composition. All powder materials show a time-dependent release process with total copper release rates less than 3 $\mu g/cm^2$ per hour at steady state conditions, for all media investigated. The importance of using relevant test media when simulating different interstitial lung conditions and difficulties encountered when comparing powder particles of essentially different properties are thoroughly discussed.
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Keywords: Copper release; Powder particles; In vitro tests; Synthetic body fluids; Simulation of interstitial lung conditions; Skin contact

1. Introduction

Human exposure to particulate matter, originating from e.g. traffic, combustion and fumes in our daily environment, has lately found an increasing interest despite being an issue of general importance during the last decades. Studies have been carried out to determine concentrations and composition of airborne particulate matter at different urban and industrial sites and working places (Liu et al., 2004; Oravisjärvi et al., 2003; Querol et al., 2004). It is well known that air pollution, in form of particles of varying composition and size range, has an impact on the respiratory health on humans and affects the lung function growth of young people in particular (Brunekreef and Forsberg, 2005; Gauderman et al., 2004; Roemer et al., 2000). The particles can include trace metals, consist of metal compounds or even be in metallic form, depending on the prevailing exposure situation (Fang et al., 2005; Harrison and Yin, 2000; Karlsson et al., 2005; Konarski et al., 2004). The effect on human health due to exposure, e.g. inhalation of metal containing particles and its mechanisms, is at present relatively unclear. It is however evident that particle size and size distribution are crucial parameters to consider. Small particulate matter ($\varnothing < 2.5$ μm) and aggregates of nano-particles that easily fragment, have been reported capable to penetrate the alveolar region of the respiratory system, causing an inflammatory response (Harrison and Yin, 2000; Murr et al., 2004). Particles with a diameter of up to 100 μm are considered inhalable (Ogden, 1992; ISO 7708, 1995).

Copper powder is used in brake linings/pads where it functions as a heat conductor since it dissipates the heat of friction.

* Corresponding author. Tel.: +46 8 790 6878; fax: +46 8 208284.
E-mail address: klarami@kth.se (K. Midander).

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Copper is also present in rubber tyres, asphalt and concrete, all contributing to the release of particles into our environment. The presence of copper in fine and coarse particles in traffic environments has recently been shown by Fang et al. (2005). The total emission of copper released from brake linings, tyres, and street/road paving from one kilometre of a main distributory street has been estimated to 1.2 kg per year (Landner and Lindeström, 1999).

Copper oxide is used e.g. in antifouling paints, wood preservatives, as pigment and as catalysts (Landner and Lindeström, 1999).

Pre-patinated copper products have become a frequent, and popular material for roofing, facing and other architectural applications. It is often used to repair naturally aged and patinated copper constructions, as well as for new constructions due to architectural and aesthetic reasons. Nowadays, decreasing pollutant levels of e.g. gaseous SO_2 in the Western world result in reduced growth rates of the greenish outermost layer of natural patina. Pre-patinated copper materials are today available with a similar greenish surface that mimics the naturally obtained greenish patina.

Artificial patina on pre-patinated copper products is produced from a paste containing e.g. different copper sulphates that is sprayed on pre-oxidised massive copper sheets. This can either be performed on site, or on the production line. When a new, pre-patinated copper roof is laid, artificial patina particles can be released from the surface as a result of handling of the pre-patinated sheet including e.g. bending and subsequent cracking. This release may cause a working environment where artificial copper patina particles can come into contact with the skin or possibly be inhaled.

Field exposures have recently been performed to assess the environmental interaction and to provide accurate data on corrosion-induced release of copper from pre-patinated copper sheet during atmospheric exposure (Odnevall Wallinder et al., 2002). Data on copper release into fluids, relevant for a human exposure to natural patina, artificial patina particles or pre-patinated flat products, are non-existent in the literature. The research presented in this paper was therefore initiated to improve the current knowledge on copper release from particles and in particular, to provide quantitative data on release rates from artificial patina particles in contact with different synthetic body fluids (PBS, artificial sweat, ALF, Gamble's solution).

Phosphate-buffered saline (PBS) is a standard physiological solution that mimics the ion strength of human blood. It is generally used in research and medical health care, e.g. injected intravenously to replace fluid after fluid loss. Artificial sweat simulates the hypo-osmolar fluid, linked to hyponatraemia (loss of Na^+ from blood), which is excreted from the body when sweating (Nilsson-Ehle, 2003). Artificial lysosomal fluid (ALF) and Gamble's solution simulate different interstitial conditions in the lung. The former simulates conditions occurring in conjunction to phagocytosis by cells, i.e. similar to an immunological reaction of the body, and the latter the interstitial fluid deep within the lung at normal health condition. The solutions have previously been used to investigate e.g. the solubility of mineral fibres, the bioavailability of cobalt-containing materials and metal release from stainless steel (Christensen et al., 1994; Herting et al., in press; Mattson, 1994; Stopford et al., 2003). The complexity in composition and difference in pH of the different synthetic body fluids investigated in this study varies as well as the respective milieu simulated.

Depending on size, shape and composition, inhaled particles may interact with cells and tissue in the lung compartment differently. The mechanisms of these interactions, affecting respiratory health, are relatively undefined or unknown. The mechanism of metal-containing particles, e.g. copper, in contact with lung tissue/cells or skin, includes the release of metal ions that may have a certain effect on the human body. Knowledge in the release process from copper-containing particles in simulated body fluids contribute to the general understanding of potential harmful effects of different particles to the human body and can be useful in further work to assess human health risks due to particles.

The aim of this study is to provide quantitative data on copper release from powder particles of different copper materials, including artificial copper patina, Cu_2O and metallic Cu, when exposed to different synthetic biological media that simulate an inhalation scenario and/or skin contact. The effect of particle size, size distribution, morphology and composition of powder particles is thoroughly discussed. Massive sheet copper products were also included, serving as reference materials for comparison of data.

2. Materials and methods

2.1. Materials

Powder particles with diameters up to 40 μm, and massive sheet products of different copper materials were included in this study (Table 1). All materials, except the artificial patina powder, are commercially available and were supplied by Outokumpu Copper.

The pre-patination process on massive Nordic Green PLUS™ (NG) involves the application of a paste composed of basic copper sulphates (27% Cu), ferric sulphate (21–23% Fe), acrylic binder material and pigments, onto pre-oxidized copper sheet. The resulting copper component of the artificial patina is mainly composed of brochantite ($Cu_4SO_4(OH)_6$) (Virtanen et al., 2002). Powder particles of the NG artificial patina, were produced by Outokumpu Copper, Pori, Finland by spraying the paste onto glass plates, dried, and manually scraped off and ground to powder particles. The size distribution of the produced particles, from sieve analysis made by Outokumpu, is presented in Fig. 1.

The specific surface area of the powder particles was measured by BET-analysis (adsorption of nitrogen at cryogenic condition) using a Micromeritics FlowSorb II 2300 instrument at Kanthal AB, Sweden.

Table 1
Materials investigated within this study

Test material	Powder particles	Massive sheet product
Artificial patina	X	
Cu_2O	X	
Cu	X	
Nordic Green PLUS™ (NG)		X
Cu		X

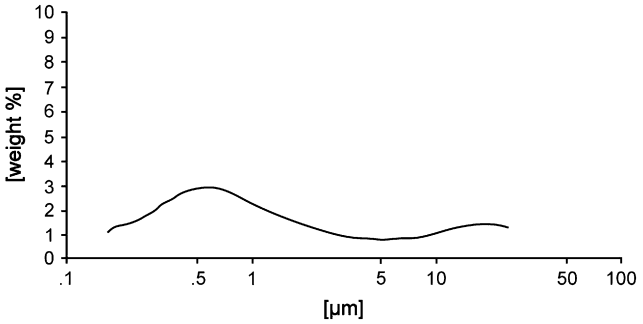


Fig. 1. Size distribution of artificial patina powder particles expressed in weight percentage of particle diameter in μm.

Triplicate powder material samples were prepared in two different loadings, 100 mg/L and 200 mg/L, the latter used only for initial tests. The particle loadings were considered relevant for simulation and selected to avoid agglomeration of particles and to ensure the contact of fresh solution to the particle surface during exposure (Midander et al., 2005). The balance used was a Mettler AT20 with a readability of 2 μg. Single-sided triplicate samples of Nordic Green and duplicate samples of copper sheet were prepared in quadratic coupons, 3 × 3 cm. To ensure a well-defined exposed surface, the backsides were covered with adhesive tape (Nitto, HD pipe wrap vinyl tape 51) and the edges were covered with wax. Reference samples of Plexiglas were prepared and exposed at identical exposure conditions to ensure that no copper was released from the wax or the tape. Generally, the materials were exposed as-received. For copper sheet, the surface was ultrasonically cleaned in acetone (3 min) and isopropanol (3 min) prior to exposure.

2.2. Test media

Immersion experiments were performed in vitro in three different synthetic body fluids and one physiological saline solution. The synthetic body fluids model certain body conditions, simulating sweat and mimicking different interstitial lung conditions, respectively. The chemical composition of each fluid is presented in Table 2. Citrate in Gamble's solution replaces proteins and acetate is added to represent organic acids (Kuhn and Rae, 1988; Moss, 1979; Stopford et al., 2003). Artificial sweat was prepared according to the EN1811 standard, stored in darkness and used within 3 h after preparation (Bianchi et al., 2000; Kanerva et al., 1994). Competence in handling and preparation of the test solutions has been gained during the last five years at the Div. Corrosion Science, KTH (Herting et al., in press; Midander et al., 2005).

Table 2
Composition (g/L) and pH of PBS and synthetic body fluids; artificial sweat, ALF and Gamble's solution (Bianchi et al., 2000; Kanerva et al., 1994; Moss, 1979; Stopford et al., 2003)

Chemicals	PBS	Artificial sweat	ALF	Gamble's solution
$MgCl_2$	–	–	0.050	0.095
NaCl	8.77	5.00	3.21	6.019
KCl	–	–	–	0.298
Na_2HPO_4	1.28	–	0.071	0.126
Na_2SO_4	–	–	0.039	0.063
$CaCl_2 \cdot 2H_2O$	–	–	0.128	0.368
$C_2H_3O_2Na$	–	–	–	0.574
$NaHCO_3$	–	–	–	2.604
$C_6H_5Na_3O_7 \cdot 2H_2O$	–	–	0.077	0.097
KH_2PO_4	1.36	–	–	–
NaOH	–	–	6.00	–
$C_6H_8O_7$	–	–	20.8	–
H_2NCH_2COOH	–	–	0.059	–
$(NH_2)_2CO$	–	1.00	–	–
$CH_3CHOHCO_2H$	–	1.00	–	–
$C_4H_4O_6Na_2 \cdot 2H_2O$	–	–	0.090	–
$C_3H_5NaO_3$	–	–	0.085	–
$C_3H_3O_3Na$	–	–	0.086	–
pH	7.2–7.4	6.5	4.5	7.4

The solutions were prepared using ultra-pure water and chemicals of analytical grades. All vessels and accessories were acid-cleaned (at least 1% HNO_3 for more than 24 h) to avoid any risk of contamination. The compounds of Gamble's solution were added following the order in Table 2 to prevent precipitation of salts. Reference samples consisting of synthetic body fluids or PBS were included for each exposure.

Despite the lack of proteins, the different synthetic biological media are relatively complicated in composition and handling. It should be stressed that they only mimic real body conditions to some extent, i.e. the complexity and function of the real body fluids are difficult to simulate. In vitro results in the synthetic biological media presented within this study can, in a simple way, provide information that could be relevant for a real situation.

2.3. Exposure conditions

All materials were exposed in acid-cleaned (at least 1% HNO_3 for more than 24 h) HD polyethylene Nalgene® jars. The vessels were filled with 50 ml solution, leaving a minimal volume of air within the jar, in particular of importance for the exposure in Gamble's solution (Herting et al., 2005). The vessels were closed and sealed with Parafilm to avoid leakage. Samples were then placed under dark conditions in a mini incubator keeping the temperature at 37 ± 2 °C and gently rocked bi-linearly at a frequency of about 25 cycles per minute. This procedure has shown to provide reproducible and relevant results for metal release studies from particles (Midander et al., 2005). Four different time periods were investigated; 0.5, 1, 4, 8 and 24 h of exposure. An additional time period after 48 h of exposure was studied for immersions in Gamble's solution, to ensure steady state conditions. After exposure, the samples were slowly cooled to ambient room temperature. Prior to separation of the copper material sample from the test solution, the pH of each sample was controlled. During the time of immersion, maximum increase in pH of 0.1 units, in the case of PBS, ALF and artificial sweat, and 0.8 units for Gamble's solution was considered acceptable (Herting et al., 2005).

Coupons of massive Cu-, and NG-sheet were carefully removed after exposure, rinsed with ultra-pure water and dried in air. The coupons were then wrapped in paper wipes (Kimberly-Clark) and stored in plastic bags in a desiccator for subsequent surface analysis. Separation of powder samples from the test solutions was made by centrifugation for 10 min at 3000 rpm. The resulting supernatant solution could then simply be poured off to a storage flask (Nalgene®, LDPE). In some cases, very fine and light particles were agglomerated and floated on the solution surface due to high surface tension of the test solution. These particles were carefully removed using acid-cleaned Pasteur pipettes before the test solution was transferred to the storage flask. Prior to analysis, the test solution was acidified using 200 μl of 65% ultra-pure HNO_3.

2.4. Metal analysis

The total concentration of released copper was analysed by AAS, atomic absorption spectroscopy (Instrumental Laboratory Model IL551). The results are based on 10 replicate readings of each sample and the calibration curves were consecutively verified during analysis. The detection limit was 0.03 mg/L. Since the copper concentration of the reference samples was generally below the detection limit, its contribution was not taken into account.

2.5. Surface analysis

The composition of the outermost surface layer was evaluated, for selected unexposed and exposed samples, with X-ray photoelectron spectroscopy, XPS (Kratos AXIS HS). All measurements were performed with a monochromatic AlKα X-ray source (1486.6 eV) operated at 300 W (15 kV/20 mA). Wide scans and detailed scans were acquired for the following elements and associated electron orbital: Cu 2p, C 1s, O 1s, S 2p, Ca 2p, P 2p, Cl 2p, and Na 1s.

The elemental peak positions on the binding energy scale provide the chemical state information.

Finally, scanning electron microscopy (SEM) was used for investigating the appearance and morphology of the different materials. SEM-analyses were performed at Outokumpu Copper (JEOL 820, Tracor Series II Microanalytical EDS system) and at St. Jude Medical (FEI Quanta 200, INCA X-sight, Oxford Instruments).

3. Results and discussion

3.1. Surface condition of test materials and surface area of powders

Specific surface areas of the three different powder particle materials, measured by BET-analysis, are presented in Table 3. It is noticeable that the measured specific surface area of the artificial patina is essentially different and more than hundred times larger compared to both powder particles of metallic Cu and Cu_2O. A large surface area on artificial patina was also indicated by the particle size distribution measurement, showing a large fraction also of small particles (<3 μm), see Fig. 1.

Morphology studies with SEM clearly show large differences in shape and morphology between the different powder particles investigated (Fig. 2). For comparison, the surface morphology of massive pre-patinated NG is displayed (Fig. 2g,h).

Ninety percent of the artificial patina powder particles ($\varnothing \leq 40$ μm) have a particle size with a diameter below 16 μm. Its shape (Fig. 2a,b) is definitely not spherical and the surface morphology and condition of the powder particles are porous and fluffy. These morphological studies clearly show the importance of surface condition on the specific surface area (14.25 m^2/g). The morphology is the result of the application technique of the green artificial patina (spraying) and the presence of binder materials within the sprayed solution. The main constituents of the outermost surface layer, as measured with XPS, are C and O from surface contamination and the binder material, and Cu(II), O^{2-}, OH^- and SO_4^{2-}, from added copper salts.

Based on SEM analysis, powder particles of Cu_2O, Fig. 2c,d show angular shapes with an approximate diameter varying typically between 10 and 30 μm. The measured specific surface area is significantly lower (0.134 m^2/g), compared to artificial patina powder particles. This is due to a substantially less porous surface with fewer, and less pronounced surface features. However, it should be noted that release rates of copper from edges and corners, potentially can be enhanced in an unpredictable way. According to XPS, the outermost surface layer is predominantly composed of copper as Cu(I) and a small fraction of Cu(II). Cu(I) is related to Cu_2O and Cu(II) probably to CuO or $Cu(OH)_2$, commonly detected on oxidized copper.

Table 3
Specific surface area of powder particles of copper measured with BET

Powder particle	BET area (m^2/g)
Artificial patina	14.2
Cu_2O	0.134
Cu	0.099

SEM observations reveal that atomised powder particles of metallic Cu are solid and spherical (Fig. 2e,f), with a diameter typically between 8 and 20 μm. These particles show the lowest specific surface area (0.099 m^2/g). As a result of a totally different manufacturing process, the morphology with pits and cavities is very different compared to Cu_2O and artificial patina. These variations in surface structure can result in differences in copper release rates. No XPS measurements were made on the Cu-powder.

The surface morphology of pre-patinated massive NG (artificial patina paste sprayed on pre-oxidised copper sheet) includes evenly scattered pores or cavities in the size range of 10–30 μm (Fig. 2g,h). These pores are the result of the presence of additives in the artificial patina paste to ensure proper surface coverage. XPS measurements indicate only a small percentage of Cu(II) in the outermost surface film, being predominantly composed of C and O from the binder and filling materials.

3.2. Effect of particle loading

Differences in copper release rates from two different particle loadings of artificial patina (100 mg/L and 200 mg/L) were initially investigated in PBS. The results are presented in Fig. 3, showing similar results without any significant deviation in release rate per area (triplicate samples) between the two loadings. A release rate without taking into account the surface area would be different between the two loadings. The similarity in results indicates no severe effect of particle agglomeration, and an efficient exposure of the powder particle surfaces to fresh solution, as a result of the gentle rocking of samples. Previous results have shown larger particle loadings to suffer from agglomeration, with lower release rates as a result. This may however not be relevant for the simulated situation (Midander et al., 2005).

Both particle loadings were considered relevant and experimentally viable. However, since a smaller loading further reduces the risk of a potential agglomeration, this loading was selected for all copper release investigations.

Moreover, Fig. 3 shows the copper release rate from artificial patina in PBS to be time-dependent. The rates are initially high (>0.05 $\mu g/cm^2$ per hour), which decreases to lower rates, and declines towards steady-state conditions after 8 h (<0.003 $\mu g/cm^2$ per hour).

3.3. Kinetics of copper release from artificial patina powder exposed to different synthetic body fluids and PBS

Copper release rates from artificial patina powder were measured in different synthetic body fluids after certain time periods during 24 h (48 h in the case of Gamble's solution to ensure steady state conditions). Kinetics data of total copper release rates for the different test media are presented in Fig. 4. All media show the same general trend, with decreasing

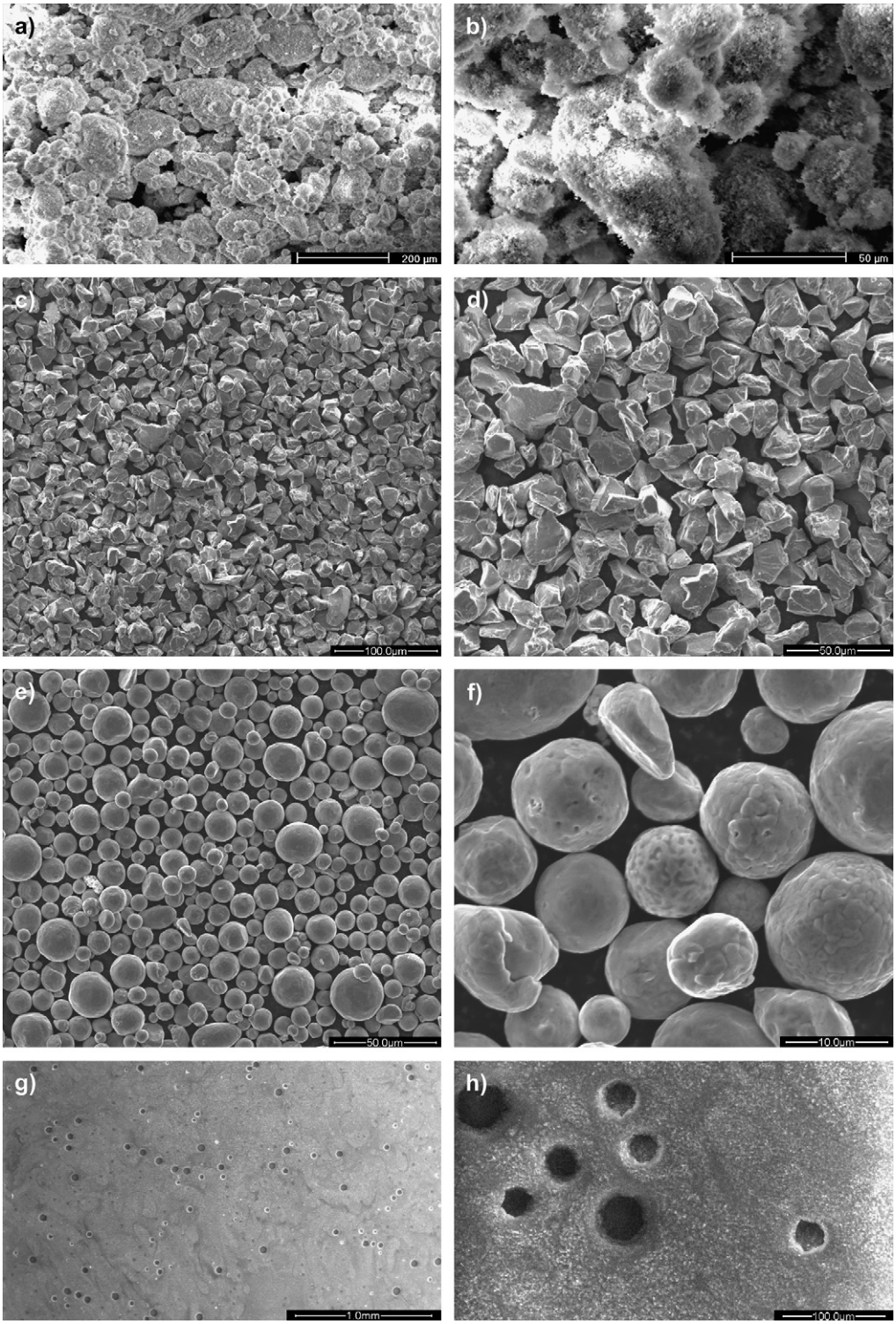


Fig. 2. Secondary electron images illustrating differences in shape and surface morphology of powder particles: (a,b) artificial patina, (c,d) Cu_2O, (e,f) Cu and (g,h) massive NG sheet.

copper release rates with time. Total release rates of copper decrease according to the following sequence: ALF > Artificial sweat ≥ Gamble's solution > PBS. The release rate in ALF is initially more than 10 times higher compared to rates in Gamble's solution, PBS and artificial sweat. The inset plot, using a logarithmic scale, visualises the difference in release rates after longer time-periods of exposure. Contradictory to findings in the other synthetic biological media, 24 h of exposure in Gamble's solution was not sufficient to reach steady-state conditions. However a relatively constant rate ($\approx$0.01 µg/cm^2 per hour) was reached after 48 h. Even though the steady state release rate in Gamble's solution is low, it is

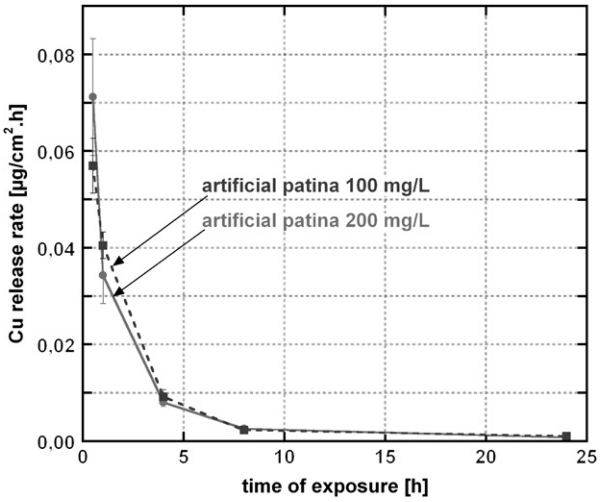


Fig. 3. Copper release rates per unit surface area in PBS from artificial patina powder particles of two different loadings, 100 mg/L and 200 mg/L, respectively.

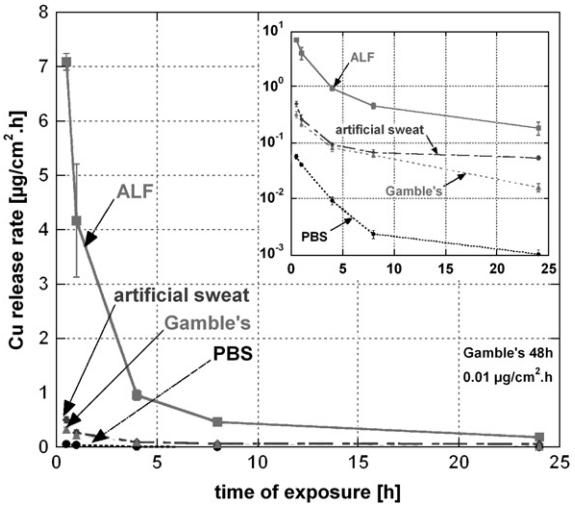


Fig. 4. Copper release rates per unit surface area from artificial copper patina powder exposed to different synthetic body fluids and PBS. The inset plot presents the results on a logarithmic scale.

approximately three times higher compared to the steady state release rate in PBS (<0.003 µg/cm^2 per hour). Since the pH of Gamble's solution and PBS is almost similar, these results imply that the more complex composition of Gamble's solution determines the release process. Gamble's solution is also a relevant media when simulating lung compartment conditions.

Steady-state release rates of total copper and corresponding concentrations are compiled in Table 4 for powders and media investigated.

3.4. Kinetics of copper release from different powder copper materials exposed to Gamble's solution

Kinetic copper release data from different powder copper materials exposed to Gamble's solution are shown in Fig. 5. Generally, decreasing release rates over time are observed for all powder materials with the highest release rate, 69 µg/cm^2 per hour, for the Cu-powder at the initial sampling period. The variation in data for the Cu-powder probably relates to the characteristics of particles of individual samples within the test triplet, e.g. differences in properties including size distribution, shape and surface layer composition and morphology. The steady state release rate for both the Cu-powder and the Cu_2O-powder reaches a value below 3 µg/cm^2 per hour, whereas the rate for the artificial patina powder reaches a level of less than 0.01 µg/cm^2 per hour, see Fig. 5 and Table 4.

Results of copper release rates for the different powder materials show that both the Cu-powder and the Cu_2O-powder release copper at a much higher rate per surface area compared to the artificial patina powder, inset plot Fig. 5. These differences are partly a result of differences in the copper content of the different powders investigated, but primarily a result of essentially different surface areas, as discussed earlier. The artificial patina powder with a significantly higher surface area comprises an unknown fraction of matrix material (binder, pigments) that is not releasing any copper. This fraction most probably contributes to a large extent to the large surface area of artificial patina, whereas its fraction of the total mass is less pronounced. Therefore and possibly, it is more correct to evaluate and compare kinetic data from different copper powder materials as a dimensionless quotient at each time period sampled, by normalizing with mass instead of specific surface area.

$$\frac{\text{mg Cu released}}{\text{mg Cu powder loaded}}$$

The results and corresponding total copper concentrations, for the exposure in Gamble's solution, are shown for the different powder materials in Fig. 6. The results show small differences in copper release between the different powder materials. Initially, the copper release decreased according to the sequence

Table 4
Steady-state release rates of total copper and corresponding concentrations for powders and test media after 24 or 48 h of exposure (Gamble's solution)

Test material		PBS (24 h)	Artificial sweat (24 h)	ALF (24 h)	Gamble's solution (48 h)
Artificial patina	(µg/cm^2/h)	0.0010 ± 0.0002	0.054 ± 0.0037	0.19 ± 0.050	0.0096 ± 0.0011
	(mg/L)	0.36 ± 0.06	20 ± 1.6	67 ± 16	6.8 ± 0.5
Cu_2O	(µg/cm^2/h)	–	–	–	2.8 ± 0.33
	(mg/L)	–	–	–	18 ± 0.94
Cu	(µg/cm^2/h)	–	–	–	2.4 ± 0.41
	(mg/L)	–	–	–	14 ± 2.5

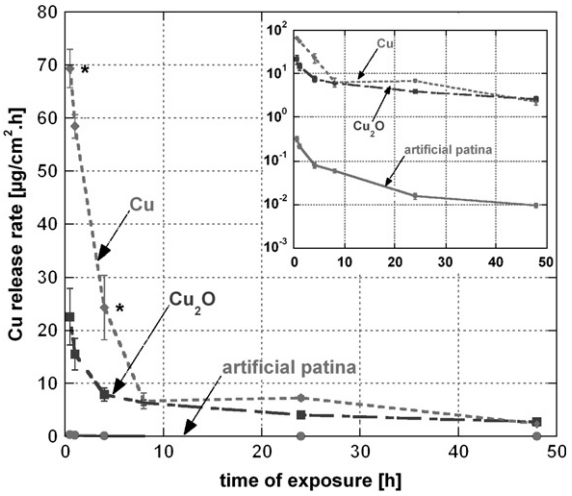


Fig. 5. Copper release rates per unit surface area from different powder copper materials; artificial patina-, Cu_2O- and Cu-powder respectively, exposed to Gamble's solution. The inset plot presents result on a logarithmic scale. Stars denote duplicate samples (otherwise triplicates).

Cu > artificial patina ≥ Cu_2O, whereas small differences were observed during steady state; Cu_2O ≥ Cu ≥ artificial patina. These small differences are also evident when examining total copper concentrations for each sampling period, see Fig. 6.

No changes in surface condition and morphology were observed after exposure of artificial copper patina particles and Cu_2O-powder particles in Gamble's solution.

Since the exposed powders have very different surface characteristics, composition, surface area and morphology, the use of the quotient noted above constitutes a useful way to compare copper release from the different powders.

3.5. Total amount of copper released from powder and massive copper materials exposed to Gamble's solution

To visualise the difference in metal release from powder material and massive sheet products, Fig. 7 displays the total amount of copper released after 24 and 48 h.

After 24 h of exposure to Gamble's solution, Fig. 7 (left), the highest amount of copper was released from the two pure copper materials (massive sheet: 0.47 mg and powder: 0.99 mg) and from Cu_2O (0.66 mg). Even though the exposed area of massive Cu-sheet was larger (geometric area of 9 cm^2, specific surface area even larger due to e.g. surface roughness), compared to the exposed area of the Cu-powder material (mean surface area of 5.71 cm^2), more copper was released from the latter. Similar results were obtained for the artificial patina powder and the NG massive sheet. However, since the exposed surface areas are significantly different, with a mean specific surface area of 719 cm^2 for the artificial patina powder and a geometric area of 9 cm^2 for massive NG of unknown specific surface area, a comparison would be erroneous. In addition, the surface conditions of the powder, being porous and fluffy, are far different from the compacted, massive surface of the artificial patina sheet material.

Calcium and phosphate were detected with XPS on all surfaces after 24 h of exposure, which may have an inhibiting effect on the copper release from the powder particles and the massive sheet materials. This effect has previously been shown for stainless steel (Herting et al., in press).

After 48 h of exposure to Gamble's solution (Fig. 7, right), the total amount of released copper increased for the Cu_2O-powder, the artificial patina and the massive NG, whereas the Cu-powder and the massive Cu-sheet exhibit a

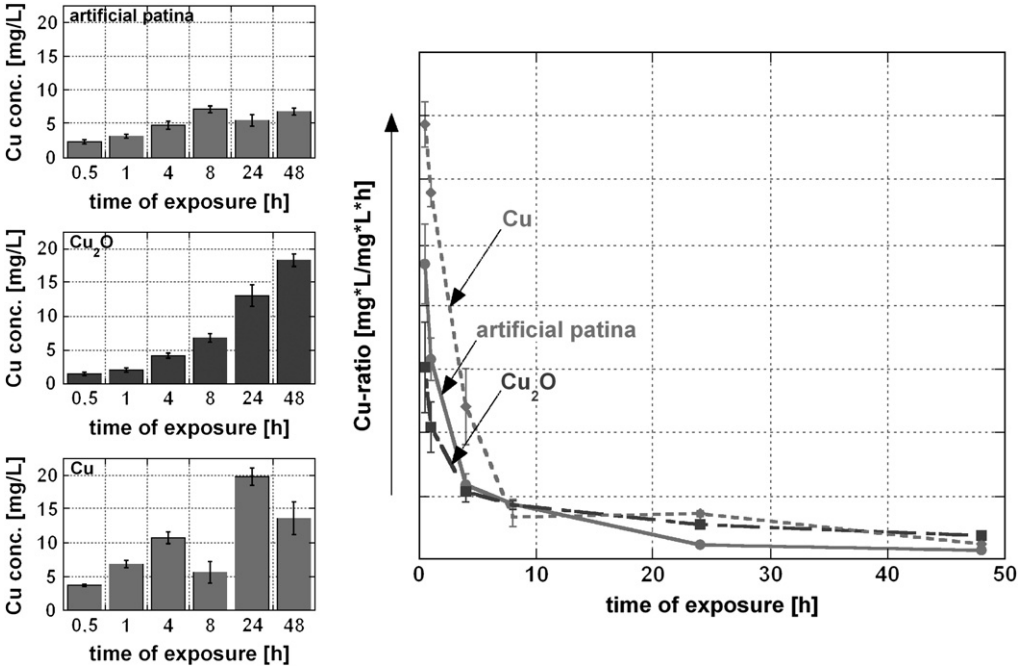


Fig. 6. Total concentrations of copper and a copper quotient vs. time of exposure for different copper powder materials exposed to Gamble's solution.

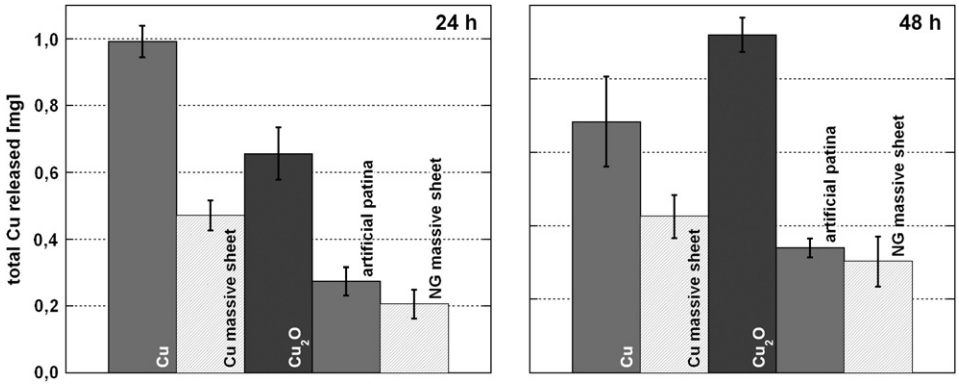


Fig. 7. Total amount of copper released from Cu-, Cu_2O-, and artificial patina powder and massive copper materials after 24 h and 48 h of exposure to Gamble's solution.

reduced released copper quantity. The reduction may be related to the formation of corrosion products of lower solubility on both powders and massive sheet, and a reduced fraction of copper in the surface layer. On the other hand, a reduced release due to formation of corrosion products can also be related to differences in surface conditions of individual particles in the powder sample.

Higher amounts of released copper from the powder materials compared to the massive form are still observed after 48 h, although the differences are less pronounced compared to 24 h.

4. Conclusions

The exposure to particles in our daily environment influences the human health since it affects respiratory function and may cause skin sensitisation. The mechanism of particle interaction with cells and/or tissue is relatively unclear, but may vary depending on the size, shape, surface properties and material of interacting particles. Quantitative copper release data from copper-containing powder particles exposed to simulated lung/skin environments obtained within this study may contribute towards improving the current knowledge of the effect of particles on the human health. Detailed data on copper release rates have been generated from various copper powder materials, including artificial patina, Cu_2O and metallic copper. In vitro studies have been performed in PBS, artificial sweat, ALF and Gamble's solution, where the latter two simulate different interstitial lung conditions. Massive sheet material of pre-patinated copper (NG) and pure copper were included for further comparison and better understanding of generated data. The following main conclusions were drawn:

- The copper release process is highly time-dependent for all powders and media investigated.
- Copper release rates from artificial patina powder were generally low. Before reaching steady state conditions copper release rates decreased according to the following sequence: < 8 μg/cm^2 per hour in ALF, <0.5 μg/cm^2 per hour in artificial sweat and Gamble's solution and <0.08 μg/cm^2 per hour in PBS. Release rates were below 0.2 μg/cm^2 per hour when reaching steady state conditions in all media investigated.
- The most important factors affecting metal release from particles are the particle size and the morphology of particles, which determines the specific surface area, the surface properties and the soluble fraction of the particulate matter.
- Due to essentially different powder particle properties and specific surface areas of the investigated materials, a comparison should not directly be made between the samples in terms of release rate per area and time unit. A more fair comparison is to establish a dimensionless ratio that rather takes into account the powder mass instead of specific surface area.
- The total amount of copper released from the Cu-powder (1 mg) was higher compared to massive sheet after 24 h of exposure, even though the exposed area of the powder samples was significantly less. Powders of artificial patina also released more copper than their corresponding massive material (pre-patinated massive NG) but the differences were less pronounced and not possible to evaluate qualitatively since the surface areas differed approximately 80 times. The pure Cu materials showed lower levels of total amount of released copper after 48 h compared to 24 h. This may be related to the formation of corrosion products of different solubility and/or deposition of Ca and P on the surface, which may locally act as a barrier for copper release. The total amount of copper released from artificial patina, massive pre-patinated NG and the Cu_2O-powder increased with time.

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