Metal release from powder particles in synthetic biological media

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Licentiate thesis

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Abstract

Humans are exposed to metals and metal-containing materials daily, either conscious, e.g. using metal tools or objects, or unconscious, e.g. during exposure to airborne metal-, and metal-containing particles. The diffuse dispersion of metals from different sources in the society, and the concern related to its potential risk for adverse effects on humans have gained an increased public and governmental attention both on a national and international level. In this context, the knowledge on metal release from metallic objects or metal-containing particles is essential for health risk assessment.

This thesis focuses on the study of metal release from powder particles of stainless steel and Cu-based materials exposed to synthetic body fluids mainly for simulating lung-like environments. The study comprises: i) development of a suitable experimental method for metal release studies of micron sized particles, ii) metal release data of individual alloy constituents from stainless steel powder particles of different particle sizes, and iii) Cu release from different Cu-based powder particles. In addition, the influence of chemical and physical properties of metallic particles and the test media are investigated. Selected results from Ni powder particles exposed to artificial sweat are presented for comparison. The outcome of this research is summarized through ten questions that are formulated to improve the general understanding of corrosion-induced metal release from metallic particles from a health risk perspective.

A robust, reproducible, fairly simple, and straightforward experimental procedure was elaborated for metal release studies on particles of micron or submicron size. Results in terms of metal release rates show, for stainless steel powder particles, generally very low metal release rates due to a protective surface oxide film, and Fe preferentially released compared to Cr and Ni. Metal release rates are time-dependent for both stainless steel powder particles and the different Cu-containing powders investigated. The release of Cu from the Cu-containing particles depends on the chemical and compositional properties of the Cu-based material, being either corrosion-induced or chemically dissolved. Moreover, the test medium also influences the metal release process. The metal release rate increases generally with decreasing pH of the test media. However, even at a comparable pH, the release rate may be different due to differences in the interaction between the particle surface and specific media.
The nature of particles is essentially different compared to massive sheet in terms of physical shape, surface composition and morphology. The surface area, and even the surface composition of metallic particles, depend on the particle size. The specific surface area of particles, area per mass, is intimately related to the particle size and has a large effect on the metal release process. Release rates increase with decreasing particle size due to a larger active surface area that takes part in the corrosion/dissolution process. The surface area that actually is active in the corrosion and metal release process (the effective area) governs the metal release process for both particles and massive sheet of metals or alloys. For particles, the effective surface area depends also on agglomeration conditions of particles during exposure.

*Keywords: metal release, stainless steel, Cu, powder particles, synthetic body fluids, test method, in vitro tests*
Preface

The following papers are included in this thesis:

I  "Elaboration of a test method for the study of metal release from stainless steel particles in artificial biological media"
K. Midander, J. Pan, C. Leygraf

II  "In vitro studies of copper release from powder particles in synthetic biological media"
K. Midander, I. Odnevall Wallinder, C. Leygraf
Environmental Pollution (2006), in press

III  "Influence of particle size on metal release from 316L stainless steel powder particles in synthetic biological media"
K. Midander, J. Pan, C. Leygraf
Manuscript

The following reports have been completed but are not included in the thesis.

"Report of a pilot study of metal release from stainless steel particles in synthetic biological media"
K. Midander, J. Pan, C. Leygraf

"A follow-up study of metal release from stainless steel powder particles in synthetic biological media"
K. Midander, J. Pan, C. Leygraf
ISSF/Eurofer report (2006)

"Nickel powder release testing in synthetic sweat"
J. Pan, K. Midander, C. Leygraf
1. Introduction

Engineering metals and alloys are widely used in the society and have brought great improvement to the quality of our daily life. Humans come in contact with metallic objects and constructions several times a day, e.g. in the home environment when cooking using cutlery, at the work place as a welder or a dentist that use their professional tools, in public places when passing the barrier entering the subway, filling up the tank, or shopping at the supermarket. In general, the contact with a specific metallic material is not something that attracts our attention since it is somehow intentional. Most contacts to metallic material do not pose any health problem, however direct contact with metal surfaces, e.g. nickel from coins or piercing jewelry, can cause contact dermatitis (Benezra et Leroy, 1988; Haudrechy et al., 1994; Hostynek et al., 2002; Kanerva et al., 1994). Moreover, and perhaps of less awareness, humans are continuously exposed to airborne particulate matter that sometimes contain metallic species.

Airborne particles are associated with a range of adverse health effects including exacerbation of asthma and chronic obstructive pulmonary diseases, as well as lung cancer and cardiovascular diseases (Brunekreef et Forsberg, 2005; Gauderman et al., 2004, Seaton et al. 1995). Traffic sources are often the main origin of airborne metal-containing particles in urban environments, e.g. wear of asphalt/paving material, rubber tires, brake/lining pads. Other sources that generate metal-containing particles are building and construction sites, as a result of, e.g., digging, founding, welding and tooling activities. Moreover, professionals in certain occupations such as workers in the metal manufacturing industry, metal refinery workers, municipal waste incinerator workers and scavenging workers may be exposed to metallic-, and metal-containing particles at elevated concentration levels (Antonini, 2003; Ghio et Devlin, 2001; Jenkins et Eagar, 2005; Kusaka et al., 1992; Sivulka, 2005; Stephenson et al., 2002; Werner et al., 1999).

The diffuse dispersion of metals from different sources in the society, the concern related to its potential risk for adverse effects on humans, and environmental effects, has gained an increased public and governmental attention both on a national and international level. This is reflected in the on-going environmental and health risk assessments on metals, e.g. Zn, Cu, Ni, initiated within the framework of the European commission, and the REACH directive on chemical substances. Several
health policies and regulations have also been developed and established. Due to lack of data, these regulations and restrictions are often conservative and based on the precautionary principle. During the last decades, limited efforts have been made to elucidate environmental effects, or health aspects related to corrosion-induced release of metals from metallic objects or metal-containing particles. Recent investigations have studied the environmental effect of corrosion-induced metal release from different copper materials, zinc-based materials and stainless steel (Bertling et al., 2006a; Bertling et al., 2006b; Odnevall Wallinder et al., 2006). However, few studies exist in the literature concerning release rates of metals from e.g. copper-based materials or stainless steels into synthetic body fluids. For metallic and metal-containing particles, such data is scarce. Reliable information on corrosion-induced metal release rates is valuable when assessing the potential risk of adverse effects arising from the exposure to metal objects or metal-containing particles.

Figure 1. Examples of different sources generating particles in the society.

This thesis provides an improved understanding of corrosion-induced metal release processes for metal particles of copper-materials and stainless steel when exposed to synthetic body fluids. The study comprises i) the development of an experimental procedure for metal release studies on micron sized particles (Paper I), ii) the
generation of metal release data of individual alloy constituents from stainless steel and copper-based materials, iii) an improved understanding of the metal release process for particles compared to flat massive sheet (Papers II, III), and iv) the importance of chemical and physical properties of metallic particles and the influence of different test media (Papers II, III).

The following 10 questions have been formulated within the context of this licentiate thesis to improve the general understanding of corrosion-induced metal release from metallic particles from a health perspective.

1. Why is data on metal release from particles of interest?
2. What differentiates a particle from a flat massive sheet?
3. How to measure metal release from particles?
4. Does the nature of different materials influence metal release from particles?
5. What impact do the test media have on metal release from particles?
6. How much metal is released?
7. What effect does particle loading have on metal release from particles?
8. How is metal release from particles influenced by particle size?
9. Does ageing of particles affect metal release?
10. What questions remain for future work?

1.1. Human exposure to particles

Several studies exist that measure the concentration of airborne particulate matter, both indoors and outdoors, in different environments including urban, industrial and rural sites, and streets of high traffic intensity. Concentrations of particulate matter with an aerodynamic diameter less than 10 µm, PM₁₀, and less than 2.5 µm, PM₂.₅, are often collected and measured, (Fang et al., 2005; Johansson et Johansson, 2002; Liu et al., 2004; Oravisjärvi et al., 2003; Querol et al., 2001). Adverse effects on human health due to human exposure to these particles have been assessed, however, the particles are often poorly characterized and their chemical composition, size distribution or shape unknown (Brunekreef et Forsberg, 2005; Fernández Espinosa et al., 2002; Gauderman et al., 2004; Harrison et Yin, 2000; Konarski et al., 2004; Machemer, 2004; Murr et al., 2004; Roemer et al., 2000).
Three main categories of airborne particles have been defined and correlated to respiratory health and area of respiratory tract: *inhalable* particles, less than 100 µm, can penetrate through the nose and mouth, *thoracic* particles, less than 11 µm, are defined as a subfraction able to pass the larynx, and *respirable* particles, less than 5 µm, able to reach the alveolar region of the deep lung (Ogden, 1992; ISO 7708, 1995). Respirable particles may cause an inflammatory response (Seaton et al., 1995; Ogden, 1992). It is postulated that nano-particles, generated within the nanotechnology industry, also may induce adverse effects on the human health. However, the mechanism of human interaction with very small particles (< 100 nm) through e.g. inhalation, ingestion or skin uptake is relatively unknown (Oberdörster et al., 2005; Seaton et al., 1995). WHO, the World Health Organization, has recently updated their air quality guidelines with an interim target (24-hour mean) for PM$_{10}$ of 50 µg/m$^3$ (WHO, 2005).

Based on current knowledge of particle toxicology, it is necessary and desirable to integrate other disciplines, such as materials science, to improve the knowledge on potential health risks, and also to provide a deepened knowledge on the role of surface and size for particle toxicity (Borm, 2002; Oberdörster et al., 2005).

1.2. **Toxicity of particles**

The mechanisms behind the effects on respiratory health due to particles are believed to include oxidative stress and inflammation. Inflammatory cells generate reactive oxygen species as a part of the immune defense against microorganisms (Donaldson et MacNee, 2001; Donaldson et Tran, 2002). It is also believed that reactive oxygen species can be formed via radicals on a particle surface, via surface bound chemicals, or via soluble transition metal components (Donaldson et MacNee, 2001; Merolla et Richards, 2005; McNeilly et al., 2004). Soluble transition metals, such as Fe, promote the generation of the toxic hydroxyl radical (OH$^-$) from H$_2$O$_2$ (Ghio et al., 1999). If hydroxyl radicals are formed close to the DNA molecule, which is possible since the electron-rich structure of DNA attracts positively charged metal ions, they may react and oxidize DNA (Dellinger et al., 2001; Knaapen et al., 2002; Pryor, 1988).

Several in vitro toxicological studies on cultured lung epithelial cells have shown different metals such as Cr, Mn, Fe and V, to be cytotoxic (Pascal et Tessier, 2004; Riley, 2005;). The predominant particle component that triggers the inflammation is
still not clear. It is however suggested that specific components need to be identified, and that mass may not be the most appropriate metric to use when assessing adverse health effects due to the exposure of particles (Ghio et Devlin, 2001). Inflammation is enhanced by coarser particles, i.e. PM$_{10}$, compared to finer fractions, probably due to higher particle concentrations of specific elements such as Fe and Cu (Brunekreef et Forsberg, 2005; Hetland et al., 2005). The exposure of cultured human lung cells to subway particles, and particles collected at street level of high traffic intensity, revealed subway particles to be significantly more genotoxic and more likely to induce oxidative stress. This was to a large extent attributed to the presence of Fe within the particles (Karlsson et al., 2005).

1.3. **Particle dissolution**

The released fraction of particles, sometimes referred to as the soluble fraction or the soluble elemental concentration (transition metals in particular), is often associated with a toxic response obtained during in vitro tests of cultivated human cells, an enhanced allergic sensitization, and/or an acute respiratory health effect (Adamson et al., 1999; Ghio et al., 1999; Hetland et al., 2001; Knaapen et al., 2002; Lambert et al., 2000; McNeilly et al., 2004; Merolla et Richards, 2005; Roemer et al., 2000). Particles from a specific environment are often collected on a filter and the toxicological effects evaluated after removal from the filter. Leaching out particles from the filter is often achieved by washing in water, or in diluted acid, during gentle (end-over-end) or intense (ultrasonic) agitation. The suspended particles are then normally separated from the leaching media by centrifugation and divided into two different fractions; the released fraction (often denoted soluble fraction), i.e. the amount that has been dissolved in the leaching media, and the insoluble particle fraction, i.e. not affected by the filter leaching media (Hetland et al., 2001; Ghio et al., 1999; Knaapen et al., 2002; McNeilly et al., 2004; Merolla et Richards, 2005; Ovrevik et al., 2005; Roemer et al., 2000). Accordingly, the released fraction of particles collected and leached from filters, is the amount of soluble species (e.g. metal ions, anionic, cationic species), into the leaching media. Recent attempts have been made to improve the removal of collected particles on filters by extracting specific chemical forms of the metals of interest e.g., metal carbonates, metal oxides or metal bound to organic matter, from particles that can be biologically available to the respiratory tract of the human body (Fernández Espinosa et al., 2002).
It should however be noticed that the released or soluble fraction of e.g. metals from particles, during particle removal from collecting filters, is due to a chemical dissolution of particles during the removal procedure, and may not reflect the real situation of corrosion-induced metal release from particles in contact with humans.

1.4. Stainless steel and copper materials

The common use of engineering alloys and metals in the society combined with an increased awareness of the potential risk of adverse health effects associated with the exposure to metallic materials through inhalation and/or skin contact, make the need for accurate metal release data evident. Accurate understanding and interpretation of such data on, e.g., metal release from particles, requires a deep understanding of material characteristics and the difference between a pure metal (e.g. Ni, Cr) and a metal alloy (e.g. AISI 316L stainless steel).

Basically, stainless steels are Fe-based alloys that contains at least 12 wt% Cr. Stainless steels possess high resistance to corrosion due to passivity, i.e. the formation of a stable and protective surface oxide film (the passive film). Even though the passive film is very thin, only about a few nm (1 nm = 10^{-9} m) thick at normal atmospheric conditions, it acts as an efficient barrier for corrosion and a potent remedy in reducing metal dissolution. The passivity phenomena have during the last decades been subject of intensive research aiming at improving and developing corrosion resistant engineering alloys for specific applications. It is now generally believed that the passive film on stainless steel consists of an inner layer of Cr- and Fe-rich oxides and an outer layer of hydroxides and/or oxyhydroxides. During exposure to an aquatic media, Fe is preferentially released and the passive film becomes enriched in Cr. An increased Cr content of the passive film improves the corrosion resistance, which is also improved with a higher degree of alloying. Nickel oxides are generally not present in the passive film, even at high bulk contents (20 wt% Ni). However, the presence of nickel in the alloy changes the microstructure, improves the corrosion resistance and promotes re-passivation. The exposure of stainless steels to aqueous environments, results in changes in the passive film composition, and metal release rates (Clayton et Olefjord, 2002; MacDougall et Graham, 2002). Even though data has recently been generated on metal release rates of e.g. individual alloy constituents from stainless steels, the mechanism for metal release is not known.
The pure metals, such as the alloying elements of stainless steel Fe, Cr, and Ni, all possess unique material properties. Generally, also pure metals react with oxygen species in the atmosphere forming surface oxides of varying protective ability. Pure Cr forms a dense passive film similar to stainless steel but with less defects. The Cr oxide/hydroxide passive film is as a potent barrier for corrosion and metal release. Nickel forms a highly defective surface film that is passive in a large range of pH, whereas the protective ability of oxides on Fe usually is relatively poor except for rather specific conditions (MacDougall et Graham, 2002).

Copper is a pure metal, frequently used in different applications in the society, and is naturally occurring in the environment. Copper is immediately oxidized in air and aqueous environments, forming different oxides. The oxide growth is accelerated by an increased humidity and/or temperature. In polluted environments (e.g. SO₂, NO₂, O₃, NaCl) or corrosive media (e.g. Cl⁻, SO₄²⁻), different copper-rich phases of varying protective ability and solubility form on the oxidized copper surface. Naturally formed patina often acts as an effective barrier for corrosion. (Landner et Lindeström, 1999).

A strategy for handling of chemicals to ensure a high level of protection for human health and environment has been adopted within the legislative framework of the European Commission. Manufacturers and industries are required to demonstrate that all chemicals (including metals), used within their production processes, are safe to use and environmentally friendly. If any component of a chemical substance is classified as dangerous, i.e. included in the list of dangerous substances, the whole chemical substance is classified as dangerous (WHO, 2001). The strategy for handling of chemicals does not take into account that metal alloys, e.g. stainless steels, often possess fundamental differences in mechanical, chemical and physical properties, and therefore can not be considered or evaluated as chemical substances. This erroneous approach, treating metal alloys as simple chemical mixtures, has recently been thoroughly demonstrated with metal release studies on stainless steels and the pure metals exposed to simulated rain events (Herting et al., 2005a).

1.5. Particles of metals and alloys

It is intuitively evident that a metallic particle is different from its massive form in terms of natural shape. However, limited knowledge exists regarding differences in their chemical and physical properties. Surface area and composition are key
parameters that govern metal release mechanisms and the biological interaction of particles. Moreover, the particle size is related to the thickness of surface oxide on particles and hence influence the metal release mechanism, figure 2.

The surface oxide film formed on a stainless steel particle differs from the oxide formed in massive stainless steel, mainly due to a different formation process, i.e. gas-atomisation at high temperature. During atomisation, oxides, rich in Cr and Mn, are locally formed on the particle surface of low-alloyed stainless steel (12 wt% Cr). At lower temperatures the remainder of the powder particle surface is covered by Fe oxide. For particles with a mean diameter above 40 µm, the oxide thickness is about 5 nm and independent of particle diameter. However, smaller particles are less oxidized and often covered with a thin layer (~1 nm) enriched in S (Nyborg et al., 1992), see figure 2.

Figure 2. The oxide film thickness on low alloyed (12 wt% Cr) stainless steel powder depends on particle size up to a diameter of approximately 40 µm (redrawn from Nyborg et al., 1992).

The distribution of various oxygen species in the surface layer and the bulk of Ni and Co powder particles has been studied by means of different heating procedures (Grallath et al., 1997). No or scarce information on the composition of oxidized copper on copper particles is available in the literature.

1.6. Synthetic biological media relevant for particle inhalation or skin contact

Several synthetic body fluids exist that mimic real body fluids to different extent. PBS (pH 7.2-7.4), phosphate buffered saline, is a standard physiological solution that mimics the ion strength of human blood. This fluid is generally used in medical health
care, e.g. it is injected intravenously to replace fluid after fluid loss. PBS is commonly used as a reference fluid for comparative purposes.

Artificial sweat (pH 6.5) simulates the hypoosmolar fluid, linked to hyponatraemia (loss of Na\(^+\) from blood), which is excreted from the body when sweating (Nilsson-Ehle, 2003). This fluid is recommended in the available standard for testing of nickel release from nickel containing products.

Two different synthetic fluids are available to mimic respiratory tract fluids, ALF (pH 4.5), artificial lysosomal fluid, and Gamble’s solution (pH 7.4), an artificial interstitial fluid. Both fluids simulate different interstitial conditions within the lung compartment. ALF simulates conditions occurring in conjunction to phagocytosis by cells, i.e. similar to an immunologic reaction of the body, whereas Gamble’s solution mimics the interstitial fluid deep within the lung at normal health conditions. Even though both fluids have a complex composition, the absence of proteins limits the possibility to mimic real body conditions.

1.7. In vitro metal-release protocols and studies in synthetic body fluids.

The OECD transformation/dissolution protocol is applicable to studies of corrosion-induced metal-release from pure metals, alloys and sparingly soluble metal compounds in aqueous media, and to assess potential aquatic toxicity of metallic particles. In short, the protocol includes the loading of various substances (metal particles) into one liter of a specified medium (pH 8) followed by a gentle agitation of the slurry using an orbital shaker, a magnetic stirring bar, or a radial impeller. Released metal concentrations are analyzed at regular time-intervals during at least 168 hours (OECD, 2001). According to the “Nickel Directive” 94/27/EC, issued by the European Commission, the total release rate of nickel must not exceed 0.5 µg/cm\(^2\)-week for any commercially available nickel-containing products that may have human skin contact. These immersion tests are performed in artificial sweat, as described by the EN1811 standard (Brugnoni et al., 2000). Respiratory health risks associated with Ni, have been assessed by the determination of the chemical speciation of Ni in ambient air using sequential extractions (Galbreath et al., 2003).

In vitro tests of metal release from various metallic materials and metal compounds, e.g. biomaterials used for implants including 316L stainless steel, Co-, and Ti-alloys, have been investigated in a range of synthetic biological media:
phosphate buffered saline, calf serum, 0.9% NaCl, artificial saliva, 1% lactic acid, 1.2% L-cysteine, 0.01% HCl, artificial interstitial-, alveolar-, lysosomal-, and synovial fluids and artificial gastric and intestinal juice (Okazaki et Gotoh, 2004; Stopford et al., 2003). It is important that the synthetic environment is relevant for in vivo conditions in terms of physical, chemical and biological properties. The test procedure must be reproducible, providing mechanisms relevant for human interactions. On the other hand, it is important to use a test solution that is not too complicated, which enables the possibility for material screening and ranking. By using simple solutions, the interpretation of results becomes more feasible, and enables the distinction between individual effects of characteristic material properties (Kuhn et Rae, 1988).

As previously discussed, there are significant gaps of knowledge related to metal release rates from metal alloys and pure metals in synthetic biological media, and no or scarce data is available on the metal release process from metallic particles. A comparison of metal release from various metallic biomaterials in a range of biological media, show that Fe is preferentially released (< 0.4 µg/cm²) from stainless steel (SUS316L), while the released amounts of alloy constituents Cr, Ni, Mo are below limits of detection after one week of exposure in PBS (Okazaki et Gotoh, 2005). This observation is in good agreement with results of metal release from various massive stainless steels exposed in different synthetic biological media studied at the Division of Corrosion Science, KTH (He et al., 2002; He et al., 2003; Herting et al., 2005b; Herting et al., in press).

Synthetic media that mimic respiratory tracts have previously been used to investigate e.g. the solubility of mineral fibers and the bioavailability of Co-containing materials (Christensen et al. 1994; Mattson, 1994; Stopford et al., 2003). Artificial sweat has widely been used for testing of Ni release from metals and alloys (Haudrechy et al., 1993; Haudrechy et al., 1994; Haudrechy et al., 1997; Kanerva et al., 1994; Lidén et al., 1998; Randin, 1988). Only limited information on the release of Cr or Cu from implant and/or dental materials exposed to e.g. different cell culture media is available in literature (Cortizo et al., 2004; Hanawa, 2004).
2. Experimental

The aim of this research work is to provide quantitative data on metal release from stainless steel and copper-containing powders when exposed to synthetic biological media. The investigation involves the following analysis: i) BET analysis (Brunauer Emmett Teller) of the specific surface area of particles, ii) total metal concentrations of Fe, Cr, Ni and Cu using ICP-MS (Inductively Coupled Plasma Mass Spectrometry) and/or GF-AAS/AAS (Graphite Furnace-/Atomic Absorption Spectroscopy) for metal release rate calculations, iii) XPS (X-ray Photoelectron Spectroscopy) studies of the elemental composition and chemical state of surface films, and iv) SEM (Scanning Electron Microscopy) studies of surface morphologies. The experimental approach is schematically described below. All details are given in Papers I-III.

2.1. Materials

A compilation of the investigated powder particle material (316L stainless steel powders of different particle size, Cu-containing powders), synthetic media, and exposure time periods is given in Table 1. The 316L stainless steel powders are denoted UF (ultrafine) and ordered I-IV with increasing particle size. More specific data on the materials is provided in each paper.

Table 1. Compilation of powder materials of different loadings exposed in synthetic biological media for different time periods.

<table>
<thead>
<tr>
<th>Powder material</th>
<th>Loading [g/L]</th>
<th>Exposure time [h]</th>
<th>Paper No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PBS</td>
<td>Gamble’s solution</td>
</tr>
<tr>
<td>316L UF</td>
<td>0.2</td>
<td>168</td>
<td>1,4,8,24,168</td>
</tr>
<tr>
<td>316L I</td>
<td>0.2</td>
<td>168</td>
<td>168</td>
</tr>
<tr>
<td>316L II</td>
<td>0.2</td>
<td>168</td>
<td>168</td>
</tr>
<tr>
<td>316L III</td>
<td>0.2</td>
<td>168</td>
<td>168</td>
</tr>
<tr>
<td>316L IV</td>
<td>0.2</td>
<td>168,336</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>168,336</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>336</td>
<td></td>
</tr>
<tr>
<td>Artificial patina</td>
<td>0.1</td>
<td>½,1,4,8,24</td>
<td>½,1,4,8,24,48</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>½,1,4,8,24</td>
<td></td>
</tr>
<tr>
<td>Cu₂O</td>
<td>0.1</td>
<td>½,1,4,8,24</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.1</td>
<td>½,1,4,8,24</td>
<td></td>
</tr>
</tbody>
</table>
2.2. **Experimental procedure**

This thesis includes the elaboration of a test method suitable for metal release studies from particles. Parameters of importance for the release process, investigated in detail during the test method elaboration, e.g. particle loading (surface area/volume ratio), particle agitation during exposure, and particle separation from the test medium after exposure, are compiled in figure 3. Detailed information is provided in Paper I.

![Diagram of investigated parameters](image)

**Figure 3.** Investigated and optimised parameters of importance for the metal release process from particles. (Paper I).

The experimental approach applied to simulate human exposure to metal particles through inhalation and/or skin contact, is schematically illustrated in figure 4. A specific amount of powder particles is exposed in a given volume of a synthetic biological fluid during a given time period. After exposure, the powder particles are separated from the test solution by centrifugation, followed by acidification prior to metal concentration analysis.
2.3. Metal analyses

Inductively coupled plasma-mass spectrometry (ICP-MS) is a technique suitable for measuring total metal concentrations at low levels (detection limits 60 µg/L for Fe, 2.5 µg/L for Ni and 4.0 µg/L for Cr). The test solution is introduced in a plasma that ionises metal atoms, detected by means of a mass spectrometer. Due to the detection technique, mass interferences, e.g. chlorides, may disturb the analysis.

Higher metal concentrations were detected by means of flame atomic absorption spectroscopy (AAS) (detection limits 9 µg/L for Fe and 30 µg/L for Cu). The test solution is introduced into a flame that atomise metals that absorbs light of a specific wavelength (characteristic for the metal analysed). The absorbance measured is
related to the metal concentration. This technique is not very sensitive to high concentrations of chlorides.

Sub-ppb concentrations (detection limit 1.5 µg/L for both Cr and Ni) of metals can be analysed with graphite furnace atomic absorption spectroscopy (GF-AAS). The principle is the same as for AAS with flame, except that the test solution is introduced in a graphite tube and heated to approximately 2000°C. This pyrolysis causes atomisation of the metal element in vapour, through which the light of a specific wavelength passes, before detected.

2.4. **Powder characterisation**

The specific surface area (m²/g) of powder particles was measured by BET analysis (Brunauer Emmett Teller). This measurement involves the adsorption of nitrogen at cryogenic conditions. The amount of adsorbed atoms is related to the surface area by the size and numbers of nitrogen atoms adsorbed on the powder surface.

Surface morphology was studied with scanning electron microscopy (SEM). In principal SEM operates by scanning the surface with a focused electron beam and an image is obtained by collecting the scattered electrons.

*X-ray photoelectron spectroscopy* (XPS) was used to study the composition and chemical state of elements in the outermost surface film (1-5 nm). A sample surface, irradiated with high-energy photoelectrons, emits inner shell electrons of atoms. Electrons, of lower binding energy than excited photoelectrons, are analysed in terms of kinetic energy related to binding energy. Each element has a characteristic spectrum of binding energies, which may be slightly shifted depending on chemical state.
3. Summary

3.1. Why is data on metal release from particles of interest?
Humans are exposed to metals and metal-containing materials daily, either consciously, e.g. using metal tools or objects, or without any special attention, e.g. exposure to airborne metal-, and metal-containing particles. The traffic environment is a general source of non-metal or metal/metal-containing particles. However, professionals in certain occupations may be exposed to more specific types of particles.

Epidemiological studies have associated a range of adverse effects on human health with airborne particles of certain size and chemical composition (Brunekreef et Forsberg, 2005; Harrison et Yin, 2000; Roemer et al., 2000, Seaton et al., 1995). Studies on toxic aspects of particles indicate the ability of e.g. soluble transition metal components of particles, to induce oxidative stress, hence damage DNA (Dellinger et al., 2001; Ghio et al., 1999; Karlsson et al., 2005; Riley et al., 2005). Consequently, the exposure to particles has become of public interest and promoted research within the framework of environmental and health risk assessment in close collaboration with metal producing industries. It has also resulted in the development of health policies and the establishment of regulations and limit values (Socialstyrelsen, 2006; WHO, 2005).

Metal-, or metal-containing particles in contact with the human body through e.g. inhalation and/or skin contact may cause adverse effects on human health. As a part of the mechanism of the human interaction, corrosion-induced release of metal ions in the case of metallic particles, is related to material properties of the particles such as chemical composition, surface morphology and passivity. Moreover, the material properties may depend on particle size.

The dissolution/corrosion process of particles exposed to body-like conditions is relatively unexplored. Metal release studies on well defined widely used metals and alloys in massive form, including stainless steel and copper materials, exposed in different biological media have recently been published in the scientific literature (Herting et al., 2005b; Herting et al., in press; Okazaki et Gotoh, 2005). However no data is available in the literature on metal release rates from particles of these metallic materials.
In all, a comprehension of metal release kinetics and mechanisms from particles exposed in synthetic biological media, and the importance of their physico-chemical properties of the particles, will contribute to an improved understanding of their potentially adverse effects on human health.

3.2. What differentiates a particle from a flat massive sheet?

The shape of a particle can be spherical, or irregular, and the size of particles may vary considerably. Metallic particles typically consist of a core with bulk composition and an oxidized surface. Particles of metal salts, such as metal oxides or metal sulphates, are often porous, fragile and fall easily apart into smaller fragments. In general, small particles tend to form agglomerated structures. The surface morphology may be smooth, or contain several defects, such as inclusions, cracks and ridges. Since particles cannot easily be cleaned, or surface oxides removed by abrasion or polishing, a controlled surface condition is difficult, or impossible to obtain. A particulate powder sample is generally composed of particles with a broad size distribution. As a result, the total surface area of all particles is difficult to estimate. In addition, different physical/chemical properties of particles, such as surface oxide thickness and composition, may change with decreasing particle size and impurities may segregate on the particle surface.

Large variations in morphology, structure and composition of the surface oxide are also evident for massive metallic materials. However, compared to particles, surface film properties can more easily be determined for flat and massive surfaces, and the surface oxide be removed, e.g. by abrasion or polishing, thereby producing a well-defined surface.

The surface area of a particle is intimately related to its size (diameter if spherical). This is different compared to flat surfaces, where the surface area primarily depends on surface finish and often is close to the geometric area. For a given amount (mass) of particles, the total geometric surface area can be estimated based on a well-defined size distribution, and the assumption that all particles have a spherical geometry. The specific surface area (area per mass, m$^2$/g) can also be measured, e.g., with BET analysis (nitrogen absorption at cryogenic conditions). The specific surface area of a powder sample increases as the diameter of the particles decreases. Consequently, the same amount (mass) of particles, i.e. the particle loading (g/L), can vary widely in
specific surface area. Ideally, metal release from differently sized particles should be constant, when normalized to the specific surface area. However, this is not the case in real conditions. Instead, the effective surface area, i.e. the electrochemically active surface area that actually takes part in the metal release process, is a crucial parameter that governs the metal release process from particles. The effective surface area is also influenced by the particle wetness and agglomeration conditions during exposure. More details are discussed in Paper III.

In all, the size-dependent surface area and composition of particles are the main parameters that differentiate particles from the flat massive sheet form. The specific surface area of particles, area per mass, has a large effect on the metal release process. However, the effective surface area, that actually is active during corrosion/dissolution, governs the metal release process for both particles and flat massive sheet of metals or alloys.

3.3. How to measure metal release from particles?

A method applicable for metal release studies from particles must be experimentally robust, easy to use, include standard laboratory facilities, and provide reproducible results. Besides, generated data must, in addition, be relevant, taking specific material properties and characteristics of the simulated environment into account.

An OECD protocol is available for transformation/dissolution studies of metals, alloys and sparingly soluble metal compounds in aqueous media. This method is recommended and used within the framework of environmental and health risk assessments of the potential aquatic toxicity of metal particles. However, for accurate metal release measurements of metal particles including metal alloy particles with passive properties (e.g. stainless steel particles) and especially for small particles of micron or sub-micron size, the protocol needs to be modified. Important parameters that need to be considered in metal release studies from particles are compiled in figure 5.

A method suitable for metal release studies from stainless steel particles has been elaborated within the context of this thesis (Paper I). The work has comprised detailed testing of parameters including particle loading, surface area/volume ratio, agitation of particles during exposure and separation of particles from the test medium after
exposure. Moreover, two different techniques for metal concentration analyses were tested and validated, ICP-MS and AAS/GF-AAS (Papers I, III). The results suggest that a small particle loading is recommended to reduce the risk of particle agglomeration. In terms of particle agglomeration and the exposure of particles to “fresh” test media, bi-linear shaking is more beneficial compared to orbital shaking. For particle separation from the test media, centrifugation is preferred compared to syringe filtration.

Figure 5. Parameters considered to have a large influence on the metal release process from particles. The effect of some parameters has been investigated and optimised, and a suitable test method for metal release studies from particles has been elaborated (Paper I).

The experimental approach has successfully been applied to particulate materials of varying composition and size: stainless steel powders of different particle sizes (Paper III), Cu-containing powders such as artificial Cu patina and Cu oxide (Paper II), pure metal powders of Cu and Ni (Paper II and NiPERA report, respectively). The investigated test media includes PBS, artificial sweat, ALF and Gamble's solution (Papers I-III). Particle loadings and solution volumes have been scaled up and modified in different ways, all providing reproducible results (Paper II).

In all, a robust, reproducible, fairly simple, and straightforward experimental procedure has successfully been elaborated for metal release studies on particles.
3.4. **Does the nature of different materials influence metal release from particles?**

Most metals and metal alloys exposed to the ambient environment form surface oxides of varying thickness, composition and protective properties. In polluted environments, the surface oxide may become degraded, partially chemically dissolved, which reduces the corrosion resistance. However, the oxide is often re-formed through different electrochemical reactions taking place at the metal-oxide interface (the corrosion process). This process involves transfer of charges and ions, which is completely different from the chemical dissolution process of the oxide that takes place at the interface between the oxide and the environment (the metal release process). The metal release rate is often significantly lower compared to the corrosion rate and strongly depends on material characteristics such as passivity, composition, thickness and microstructure of surface oxides.

Depending on degree of alloying, stainless steels possess high corrosion resistance in many environments. The passive layer is though sensitive to local corrosion attacks and may be chemically dissolved in severely acidic environments. Total metal release rates per unit surface area and hour are presented in figure 6a for ultrafine (UF) and coarse (IV) stainless steel powder particles and massive sheet (grade 316L) after one week of exposure in Gamble’s solution (pH 7.4). Extremely low total metal release rates indicate that the surface film (in this case also adsorption of inhibiting species in the test medium) practically prevents any noticeable dissolution or corrosive attack. Higher release rates of copper (note the logarithmic scale on y-axis, figure 6b) are observed for different powder copper materials exposed to Gamble’s solution during 48 hours. The reason is that these materials do not form a surface oxide film as protective as the passive film on stainless steel. Significantly higher release rates of copper from the pure Cu powder and Cu₂O powder particles compared to the artificial patina powder particles (primarily Cu₄SO₄(OH)₆), are mainly due to a higher copper content. (In the case of Cu₂O, the release of Cu is primarily a result of chemical dissolution.) Differences in chemical and compositional properties of the artificial patina compared to the Cu₂O-, and the pure Cu powder material are hence crucial for the metal release process.
In all, differences in chemical and compositional properties of pure metals, metal alloys and different metal compounds, e.g. salts or oxides, are crucial for the metal release mechanism, i.e. chemical dissolution and/or a corrosion process. The type of material strongly influences the metal release from particles.

3.5. What impact do the test media have on metal release from particles?

Metal release data from metals and alloys, including e.g. stainless steel in massive form, exposed in a range of synthetic biological media is available in the scientific literature. However, the exposed materials are often poorly characterized in terms of e.g. surface composition and surface finish, which are critical parameters for accurate interpretation of generated data. No data can be found on metal release from well-defined metallic particles exposed in synthetic biological media.

Metal release in several synthetic biological media of varying composition and acidity, but without proteins, relevant for particle inhalation (ALF and Gamble’s solution), and/or skin contact (artificial sweat), have been investigated within the context of this thesis (Papers I-III). PBS has in addition been used as a reference. It should be stressed that the complexity and function of real body fluids are difficult to simulate.

Differences in total metal release rates per unit surface area and hour are illustrated in figure 7a for ultrafine (UF) and coarse (IV) stainless steel powders (grade 316L) exposed during one week in ALF (pH 4.5) and Gamble's solution (pH 7.4),...
respectively. The results demonstrate a significant effect of media on the metal release rate. The effect is also illustrated in figure 7b for artificial copper patina powder exposed during 24 hours in ALF, Gamble’s solution, PBS (pH 7.2-7.4) and artificial sweat (pH 6.5), respectively. The general trend is that the total release rate of metals increases with increasing fluid acidity according to the following sequence: ALF > artificial sweat > Gamble’s solution, PBS. Despite a similar pH of PBS and Gamble's solution, the release rate is higher for the artificial patina powder exposed in Gamble's solution. This is probably related to a more complex composition of the Gamble's solution. Gamble’s solution has previously shown to inhibit metal release rates from stainless steels in flat massive sheet form (Herting et al., 2005b), due to adsorption and/or precipitation of calcium and phosphate species on the surface. This implies that specific interactions between the particle surface and the test media also play an important role in the metal release process.

Figure 7. a) Total metal release rates from ultrafine (UF) and coarse (IV) stainless steel powder particles (316L) after 168 hours of exposure in ALF and Gamble’s solution, and b) Total copper release rates from artificial patina powder exposed during 24 hours in ALF, artificial sweat, Gamble's solution and PBS.

In all, the metal release rate increases generally with decreasing pH of the test media. However, even at a comparable pH, the release rate may be different due to differences in the interaction between the particle surface and the specific test media.

3.6. How much metal is released?
Large differences in total release rates have been presented within the context of this thesis for powder particles of stainless steel (0.03-2.5 µg/cm²-week) and copper-
containing materials (0.001-2.8 μg/cm²-hour) exposed to synthetic body fluids of varying composition and pH (Papers II, III).

Metal release rates from particles can be normalized to the particle load (per mass) or to the specific area, giving different perspectives for comparison. Exposed in more aggressive media, e.g. ALF, the total release rate from stainless steel particles increased with decreasing particle size (Paper III). For the stainless steel powder particles investigated, the highest metal release rate per unit surface area was measured for ultrafine stainless steel powder particles, grade 316L, exposed during one week in ALF. Assuming a spherical geometry, a particle size of 2 μm and homogeneous bulk composition of particles, the release rate can be transformed to an estimate of the degree of particle reduction. A one-week exposure in ALF (pH 4.5), with a particle loading of 0.2 g/L, resulted in a reduction of the particle diameter with 0.01335 μm (Paper III), i.e less than 0.7% of the particle size was dissolved into solution, corresponding to a removal rate of approximately 66 monolayers (1 monolayer equals 10⁻¹⁰ m). For comparison, 4.6 monolayers are removed from massive stainless steel grade 316L when exposed at identical conditions. Nevertheless, the weekly released amounts of individual alloy constituents from the ultrafine powder, was 0.037 and 0.0017 mg for Fe and Cr, respectively. These amounts are significantly lower compared to the recommended daily intake of Fe (9-15 mg) and Cr (0.10 mg) (Livsmedelsverket, 2004).

The highest release rate of Cu was measured from artificial patina exposed during 24 hours to ALF at a particle loading of 0.1 g/L. This exposure corresponds to a daily release of 0.7 mg Cu, similar to the recommended daily intake (0.9 mg) (Livsmedelsverket, 2004). For comparison, a human adult body contains on an average approximately 72 mg Cu, and a 90 g chocolate bar typically 0.18-0.75 mg Cu (Landner et Lindeström, 1999; CDA, 2001).

In all, metal release rates from stainless steel powder particles are generally very low and the weekly amounts of released metal are often far below recommended daily values for human intake, even in the most aggressive synthetic biological media investigated. Total daily amounts of Cu released from different Cu-containing powder materials were similar to the recommended daily values for human intake.
3.7. What effect does particle loading have on metal release from particles?

Powder particles with a diameter less than 10 µm (PM$_{10}$) can pass the larynx and reach the lung compartment when inhaled. Only small particles (<5µm) can penetrate the alveolar region of the lung and potentially cause an inflammatory response. It is believed that this penetration results in a reduced airflow in the outer branches of the alveolar tree. This leads to particle sedimentation in this region, and an enhanced amount of particles in contact with lung tissue. Most particles, and the main part of interacting particles that enter the lung compartment, can leave this region through the digestive system. However, the residence time of particles in respiratory tracts before any further transfer into the digestive system is unknown.

The amount of particles that can have human skin contact is perhaps easier to estimate, even though it may vary in a large range depending on the prevailing exposure situation, e.g. specific occupational exposure.

Since surface area of particles depends on particle loading, which may affect the corrosion/dissolution of particles, it is important to investigate the effect of particle loading on the metal release process.

Metal release from metals and metal alloys in massive form are often expressed as released metal concentrations. This is plausible at a given surface area to solution volume quotient and often used to assess environmental and health aspects. However, this unit is not applicable on metal particles of varying particle loading.

The importance, and effect of particle loading, i.e. the amount of particles per test solution volume [g/L], on the metal release process was investigated for powder particles of stainless steel and artificial copper patina exposed in PBS (Papers I, II). A more extensive study was made for Ni powder particles of five different loadings exposed in artificial sweat (NiPERA internal report, not included within this thesis), figure 8.

By using released metal concentrations without taking into account the particle loading, the general trend is that the released concentration increases with increasing particle loading, figure 8a. However, by normalizing the released amount of metal with corresponding particle loading and time unit, it is obvious that no clear correlation can be seen between the particle loading and the metal release rate, figure 8b. At a given test volume, different loadings result in different surface areas of powder particles exposed to the test media, and hence different quotients between the test solution volume and the exposed area. Accordingly, a lower particle loading has a
smaller surface area in proportion to solution volume, which is favorable for chemical dissolution. Moreover, a higher particle loading may exhibit a certain degree of agglomeration during exposure. For accurate interpretations, and to enable a comparison between different particle loadings, it is hence essential to determine release rates related to particle loading (or surface area) rather than only consider the released metal concentration.

Figure 8. Effect of particle loading illustrated for nickel powder particle exposure in artificial sweat during 12 hours: a) concentrations of Ni released and b) accumulated amount (µg) of Ni per mg Ni powder loaded as a function of exposure time for five different particle loadings.

In all, released metal concentrations increase with increasing particle loadings due to larger specific surface areas exposed to the test media. The released metal concentration, without considering the total particle loading, cannot be used for comparative purposes and is not sufficient to assess adverse health effects on humans.

3.8. How is metal release from particles influenced by particle size?

Differences in metal release rates of individual alloy constituents (Cr, Ni, Fe) from stainless steel powders (grade 316L) of varying particle size are illustrated in figure 9 after one week of exposure in ALF (pH 4.5). All rates are expressed as the released amount of individual metals normalized to the stainless steel particle loading. Generally, the ultrafine (UF) powder exhibits the highest amount of released metals for all alloy constituents. Fe is preferentially released from all powders (note the differences in y-axis scales), similar to previous findings on massive stainless steel (Herting et al, 2005a). Significantly higher metal release rates from the ultrafine powder, compared to the other powders, are primarily related to a notably larger
specific surface area (0.700 cm$^2$/g compared to 0.069-0.294 cm$^2$/g) (Paper III). This implies that the effective surface area, i.e. the electrochemically active surface area of particles taking part in the corrosion/passive dissolution process, is larger on smaller particles compared to coarser particles.

Figure 9. Metal release rates expressed as the amount (µg) of an individual alloy constituent released from a specific amount of stainless steel particles (1 mg) exposed one week in ALF.

However, when the release rates are normalised to the specific surface area, the release rate of Fe increases with decreasing particle size, and is considerably higher for the ultrafine powder compared to the massive sheet (Paper III). One explanation could be that smaller particles have a more “active” surface compared to larger particles, due to a thinner surface oxide film and an enhanced segregation of impurities to the surface (Nyborg et al., 1992).
In all, particle size has a large influence on the metal release process. The release rate increases with decreasing size due to a larger active surface area that takes part in the corrosion/dissolution process. A more “active” behaviour of small particles of stainless steel may be a result of a thinner surface oxide with an enhanced tendency for surface segregation of impurities.

3.9. Does ageing of particles affect metal release?

The surface condition of a material is crucial for the metal release process. The surface film formed on metal and alloys usually changes slowly when aged in air, thus ageing may affect the metal release process, as observed for massive stainless steels. A massive sheet surface can either be abraded, and/or polished in order to obtain a “fresh” surface that is well defined and reproducible. However, this procedure is not applicable to powder particles. The effect of differences in oxide thickness and composition due to ageing of particle surfaces is unexplored from a metal release perspective.

An attempt to study the effect of ageing on the surface oxide on stainless steel particles was performed within the framework of this thesis. The metal release process was therefore investigated from two as-received stainless steel powders of the same grade and similar in particle size, but different in production and storage time; powder III (denoted fresh), and powder IV (denoted aged). Both powders were annealed in an argon atmosphere by slowly increasing the temperature with 20°C per minute up to 900°C. This temperature was held constant for 1 hour to reduce oxides on the powder particle surfaces to some extent. Limited XPS and SEM investigations indicate that vacuum annealing reduced the Fe oxide on the powder particles and the surface morphology was slightly different compared to the as-received powders. However, this annealing did not result in any considerable difference in the metal release rates during exposure in ALF (pH 4.5), figure 10.
In all, even though vacuum annealing in argon reduces Fe-oxides within the surface oxide of stainless steel powder particles, it does not affect the metal release rate of individual alloy constituents compared to as-received powder particles. Surface ageing seems to have a small influence on the metal release process, at least in synthetic biological media of high acidity.

3.10. What questions remain for future work?

The relevance and applicability of the in vitro test method for metal release studies of particles, established within this licentiate work, should be further tested for other groups of materials, synthetic fluids, and smaller particle sizes. The experimental procedure currently comprises several generous simplifications, such as test fluids without proteins, particle loadings that may not be relevant for real conditions, and fabricated particulate materials that may be very different from “real” particles collected at a specific exposure site.

Powder particles subjected to metal release studies must be well characterized in terms of e.g. surface condition and composition, and particle size. Due to the small particle size and large variations between powder particles, such measurements are difficult to make. The effective surface area of particles, i.e. the electrochemically active surface area that actually takes part in the metal release process, is yet impossible to measure accurately. The electrochemically active surface area on massive samples can be estimated from electrochemical impedance spectroscopy.
measurements. On-going research at the Division of Corrosion Science, KTH, investigates the possibility to apply this technique also on powder particles.

Atomic force microscopy (AFM) may become a useful tool to characterize surface properties of powder particles. The AFM technique provides the possibility for studies of particles in situ. Limited research efforts using Scanning Kelvin Probe Force Microscopy, SKPFM, have been performed to estimate the relative nobility of different stainless steel particles, and particles of varying grade. However, more research is required.

Nanotechnology is a research area in vogue, and many new products in the society are developed based on nanoscience. This has induced national strategies for assessment of risk related to nanotechnology in several countries. Potential adverse health effects of nanoparticles are relatively unexplored, and the mechanisms of metal release from really small (submicron) metal particles suffer from significant gaps of knowledge.

It would be beneficial to combine, and correlate, metal release studies from powder particles in synthetic biological media with other in vitro methodologies such as cytotoxicity investigations on e.g. lung cells. This kind of future work, which combines interdisciplinary research activities, is essential for an improved understanding of the human interaction of engineering alloys and pure metals in particles of varying size and composition that e.g. can be inhaled or have human skin contact. An improved understanding is of importance in the assessment of adverse health aspects related to human exposure to metal-containing particles.

In all, several key questions, such as an improved material characterization, a relevant particle loading, and the relevance of test fluids in terms of composition, remains to be addressed for a thorough understanding of the metal release process from particles. Parallel interdisciplinary research activities on e.g. human toxicology, are essential to address issues related to potential adverse effects on humans.
4. Main conclusions

Experimental procedures for study of metal release from particles has been elaborated and established. Furthermore, the metal release process was investigated for stainless steel powder particles of different particle sizes and different Cu-based powder particles. The nature of particles, in terms of physical shape, surface composition and morphology, is essentially different from flat massive sheet and intimately related to the metal release process. Moreover, the influence of different test media on metal release from powder particles was elucidated. Main conclusions drawn from this investigation is shown in figure 11. Detailed results are provided in Papers I-III.

Figure 11. Main conclusions from the studies of metal release from stainless steel powder particles and Cu-based powder materials.
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