



Metal bioaccessibility in synthetic body fluids – A way to consider positive and negative alloying effects in hazard assessments

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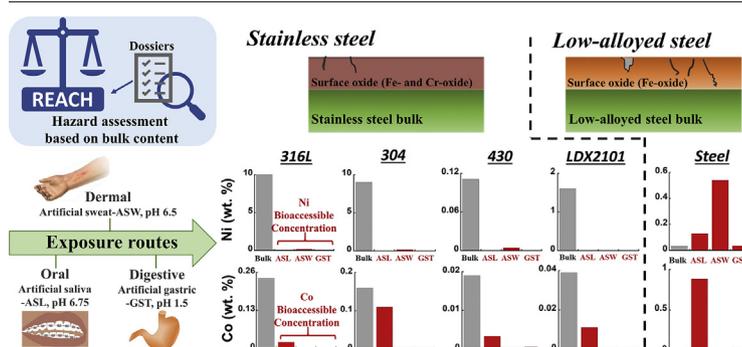
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HIGHLIGHTS

- Ni and Co release (bioaccessibility) of stainless steel and low-alloyed steel.
- Lower bioaccessible concentration than bulk content for stainless steels.
- Higher bioaccessible concentration than bulk content for low-alloyed steel.
- Metal release was highly related to surface oxide and corrosion-resistance.
- Bioaccessibility (bioelution) can refine alloy hazard classification.

GRAPHICAL ABSTRACT



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ABSTRACT

Hazard classification of metal alloys is today generally based on their bulk content, an approach that seldom reflects the extent of metal release for a given environment. Such information can instead be achieved via bioelution testing under simulated physiological conditions. The use of bioelution data instead of bulk contents would hence refine the current hazard classification of alloys and enable grouping. Bioelution data have been generated for nickel (Ni) and cobalt (Co) released from several stainless steel grades, one low-alloyed steel, and Ni and Co metals in synthetic sweat, saliva and gastric fluid, for exposure periods from 2 to 168 h. All stainless steel grades with bulk contents of 0.11–10 wt% Ni and 0.019–0.24 wt% Co released lower amounts of Ni (up to 400-fold) and Co (up to 300-fold) than did the low-alloyed steel (bulk content: 0.034% Ni, 0.015% Co). They further showed a relative bioaccessibility of Ni and Co considerably less than 1, while the opposite was the case for the low-alloyed steel. Surface oxide- and electrochemical corrosion investigations explained these findings in terms of the high passivity of the stainless steels related to the Cr(III)-rich surface oxide that readily adapted to the fluid acidity and chemistry.

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1. Introduction

A regulation of the European Union (EU), “Registration, Evaluation, Authorisation and Restriction of Chemicals” (REACH), first entered into force in 2007, aiming to protect human health and the environment from risks posed by chemicals (including metals and alloys) [1]. Under

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REACH, companies such as manufacturers and importers must ensure that substances and products placed on the market in the EU are safe, from a health and environmental perspective. In REACH, metals are typically described as substances for which registration is required. Such registration dossiers include, in addition to physico-chemical characteristics, information on toxicological hazards, aspects that are related to the bioaccessibility of metals under specific exposure scenarios. Bioaccessibility is defined as the amount of released and potentially available metal species under surrogate physiological conditions [2]. Registration dossiers are not required for alloys, such as stainless steel, as metal alloys, from a legislative perspective, are considered as mixtures of metals. It is, hence, the hazard identifications and classifications that are based on the intrinsic properties of individual metal constituents that determine the classification of an alloy, unless there is an alloy- or alloy group-specific dossier available. The same approach is also explicitly applied within the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) [3]. According to the Classification, Labelling and Packaging Regulation (CLP), nickel (Ni) metal is classified as a skin allergen (Skin Sens.1), suspected of causing damage to organs (STOT RE 1) and suspected of causing cancer (Carc. 2) [4]. Therefore, for example, alloys containing more than 1 wt% Ni have to be classified for skin sensitisation under CLP [5]. Per October 1st, 2021, cobalt (Co)-containing products with a bulk content exceeding 0.1 wt% would be classified as Carc. 1B [6]. This threshold would, hence, basically apply to most stainless steels, in which Co often is present as an impurity. Co remains in the melt during smelting of steel and therefore cannot be removed in an economically viable way.

Stainless steel, a passive iron-based alloy containing chromium and typically other alloying elements, is widely used in a broad range of applications related to sensitive environments, such as in food manufacturing and in the human body. As all materials, stainless steel is not completely inert and small quantities of metals may be released, depending on environmental conditions [7–9]. The release of metal ions/species can, from a health perspective, be either positive (for essential and bioavailable metal species at certain concentrations/doses), neutral, or negative, when exceeding threshold values resulting, for example, in allergic or genotoxic effects [10–12].

Although it is known within the materials science community that alloys often possess unique properties that are not determinable from the intrinsic characteristics of their individual metal constituents, bioaccessibility and toxicological data for alloys are scarce. Recent studies highlight the importance of surface properties of alloys and show that the applied read-across from the bulk content of constituent metals of alloys and the pure metals often is highly erroneous, especially for alloys with significant amounts of chromium and high corrosion resistance [7,9,13–16]. Released levels of metals (such as Fe, Ni, Cr, Mn and Mo) from stainless steels in given conditions are generally reported to be lower than expected from their bulk content and mainly governed by physico-chemical properties and passivity of surface oxides [7]. However, a recent study on the release of Co, lead (Pb), Ni, and copper (Cu) from different copper alloys into artificial gastric and saliva solutions showed that the release was predictable from the bulk composition [17], which has been claimed to result from their lower corrosion resistance as compared to stainless steels. The bioaccessibility of Cr (III) and Cr(VI) from stainless steels and chromium-containing alloys in different synthetic body fluids has also been investigated [18–20]. The release of Cr(VI) from stainless steel without any applied potential was found to be non-detectable (detection limit of 10 ng/L) in these solutions of pH 1.5–8.0 [18–22].

Bioaccessibility (bioelution) in-vitro testing [2,16] is a scientifically-based methodology to determine the extent of metal release from metals and alloys in synthetic biological fluids, with the advantage of being simple, rapid and reproducible, and excluding the need for animal testing. Human exposure scenarios simulated in bioaccessibility studies include, for example, ingestion and oral routes (saliva-, gastric-, intestine fluids), inhalation (lysosomal-, serum-, alveolar fluids), dermal

contact (sweat-, tear fluids) and internal implantation (lysosomal fluid) [7]. For dermal contact, a reproducible test procedure was recently elaborated for metals and alloys [23], based on a standardized test method (EN 1811) for products [24]. The elaborated method can be applied to different exposure conditions to generate bioaccessibility data for a given substance of relevance for hazard identification, classification, or for prioritisation of materials for in-vivo testing, independent of the transport and storage history of the alloy/metal surface of interest.

The main objective of this study was to generate bioaccessibility data for Ni and Co released from several stainless steel grades, a low-alloyed steel and Ni and Co metals into three synthetic biological fluids (artificial sweat - dermal, artificial saliva - oral, artificial gastric fluid - digestive). Generated data were correlated with findings on surface composition and corrosion resistance under different exposure conditions, with the aim to improve and refine hazard identification and classification of alloys.

2. Method

2.1. Materials and surface preparation

All metals and alloys were supplied by Eurofer, the European Steel Association, Brussels, Belgium. The nominal compositions of the four stainless steel grades (austenitic grades - 304 and 316 L, ferritic grade - 430, and duplex grade - LDX2101), the low-alloyed steel and the pure metals (Ni and Co), all in massive (sheet) forms, are shown in Table 1. All selected stainless steels are relevant for both the market and simulated exposure scenarios. Although the low-alloyed steel is not relevant for the same exposure environments as the stainless steels, it is a relatively common steel, for example used in construction, and therefore representative of a carbon or low-carbon steel. The most relevant exposure route for this steel is dermal contact. For reference, data on Ni and Co metals for the dermal exposure route are based on findings from a previous study [23].

The materials were prepared according to a previously elaborated method [23] to obtain uniform and comparable surface conditions for reproducible tests and to simulate as-received surface conditions as closely as possible. In short, the surfaces were ground (1200 SiC grit), cleaned (ultrasonically 5 min in ethanol and acetone, respectively), and stored in dry conditions for 24 h before being immersed for 2 h in the test fluid of interest (for passivation), followed by immersion or electrochemical testing in a fresh test fluid (c.f. Sections 2.2 and 2.6).

2.2. Bioelution testing

In vitro metal release investigations were performed in artificial sweat (ASW, pH 6.5), artificial saliva (ASL, pH 6.75), and artificial gastric solution (GST, pH 1.5). These synthetic body fluids were selected to simulate potential human exposure routes, including skin contact (ASW) and oral exposure via the mouth (ASL) or ingestion into the gastrointestinal tract (GST) [15,24,25]. GST, a diluted HCl solution, was less relevant for exposure of the steels, but relevant to simulate an exposure environment of relevance for crevice corrosion and localized corrosion, such as small crevices in implant materials [26,27]. It can, hence, be considered a worst-case environment. Although the synthetic test fluids only simulate physiological conditions to a limited extent, such in vitro results can, for the purpose of hazard assessment, provide information that could be relevant for a real situation. The chemical compositions of the biofluids and exposure conditions are given in Table 2. Ultrapure water (resistivity of 18.2 M Ω cm, Millipore, Sweden) was used as the solvent and all chemicals were of analytical grade. The pH values of ASL and ASW were adjusted by the addition of 0.5 wt% NaOH. The pH of all fluids was measured using a pH meter (PHM210 Standard pH Meter, MeterLab®, Radiometer Analytical SAS, France). ASW was freshly prepared on the day of test initiation, while ASL and

Table 1
Nominal bulk composition (wt-%) of the stainless steel and steel alloys and the pure metals based on supplier information.

| Grade | | C | Mn | Ni | Cr | Mo | S | N | Co | Fe |
|-------------------------------|---------|-------|------|--------|-------|--------|--------|-------|---------|------|
| Austenitic SS | 304 | 0.038 | 1.2 | 9.0 | 18 | 0.36 | 0.0029 | 0.039 | 0.16 | 70 |
| | 316 L | 0.017 | 1.3 | 10 | 17 | 2.0 | 0.0006 | 0.052 | 0.24 | 68 |
| Ferritic SS | 430 | 0.034 | 0.32 | 0.11 | 16 | 0.015 | 0.0016 | 0.032 | 0.019 | 83 |
| Duplex SS | LDX2101 | 0.023 | 4.8 | 1.6 | 21 | 0.28 | 0.0010 | 0.22 | 0.039 | 71 |
| Low-alloyed Steel (Reference) | Steel | 0.046 | 0.22 | 0.034 | 0.017 | <0.002 | 0.0080 | N/A | 0.015 | 99.6 |
| Pure metals (Reference) | Ni | N/A | N/A | 99.995 | N/A | N/A | N/A | N/A | <0.0001 | N/A |
| | Co | N/A | N/A | 0.018 | N/A | N/A | N/A | N/A | 99.9 | N/A |

N/A – not data available; SS – stainless steel.

GST were kept in the refrigerator (4 °C) for at most 1 day prior to the immersion tests.

Experimental details are given in [23] and in Table 2. The longest immersion period (168 h) is not relevant for any real exposure, but chosen to enable comparison with existing metal release/dissolution data and the EN1811 standard in ASW [24].

2.3. Metal release measurements

Total amounts of released Ni and Co from each specific material and synthetic fluid were analyzed by means of atomic absorption spectroscopy (Perkin Elmer AAnalyst 800) either in the graphite furnace mode (GF-AAS, for concentrations lower than 0.3 mg/L), or in the flame mode (flame-AAS, for concentrations higher than 0.2 mg/L). If the sample concentration was outside the calibration range, it was re-analyzed in a different mode.

The results are presented in the units $\mu\text{g}/\text{cm}^2$, that is, the released amount of Ni or Co per surface area of the test coupon, Eq. (1):

$$\text{Released amount} \left(\frac{\mu\text{g}}{\text{cm}^2} \right) = \frac{(C_{\text{sample}} \left(\frac{\mu\text{g}}{\text{L}} \right) - C_{\text{blank}} \left(\frac{\mu\text{g}}{\text{L}} \right)) * V(L)}{A(\text{cm}^2)} \quad (1)$$

where V is the exposure volume, A is the geometrical surface area of the test coupon, C_{sample} is the measured sample concentration of Ni or Co in solution, and C_{blank} is the measured corresponding blank (background) concentration in solution. For each test condition, the released amounts from the triplicate samples with the corresponding blank value subtracted were averaged and their standard deviation was determined (shown as error bars in the figures). Further measurement details are given in the supplementary material.

2.4. Joint expert speciation modelling

Chemical speciation modelling in the three synthetic body fluids was conducted with the Joint Expert Speciation System (JESS, version 8.7) [28]. The JESS database was firstly inspected for available reactions

Table 2

Experimental conditions: chemical compositions of the synthetic body fluids (g/L), agitation, exposure temperature and immersion periods.

| Conditions | | ASL - pH 6.75 | ASW - pH 6.5 | GST - pH 1.5 |
|----------------------------|---|---|-----------------|-----------------|
| Chemical composition (g/L) | NaCl | 0.4 | 5.0 | – |
| | KCl | 1.21 | – | – |
| | (NH ₂) ₂ CO | 1.0 | 1.0 | – |
| | NaH ₂ PO ₄ ·2H ₂ O | 0.78 | – | – |
| | Na ₂ S·9H ₂ O | 0.005 | – | – |
| | CH ₃ CHOHCO ₂ H | – | 1.0 | – |
| Agitation | 25% HCl | – | – | 4.0 |
| | | Bi-linear shaking (12° inclination, 22 cycles/min) | | |
| Exposure temperature | | 37 °C | 30 °C | 37 °C |
| Immersion periods | | 316 L - 2, 4, 8, 24, 168 h | | |
| | | Other materials - 4, 168 h | | |

between Co and Ni ions and species of the test fluids (urea, lactate, sulfide, and phosphate). In total, five reactions including Ni²⁺ and three reactions including Co²⁺ were found. The number of found and included solids (all of which were allowed to precipitate and dissolve) was 14, 18, 14, 18, 18, and 22 for Ni in GST, Co in GST, Ni in ASW, Co in ASW, Ni in ASL, and Co in ASL, respectively. The following details were obtained from / displayed by the software's record of 'Conditions, Methods and Assumptions': i) in no case were species or primitives ignored; ii) damped Newton-Raphson, with the convergence criterion of either a smaller sum of absolute equation values of 0.0 or each delta[log(unknown)] smaller than 0.0001, was the equation-solving algorithm in all cases; and iii) all equations were successfully solved {maximum iterations of 200, maximum delta[log(unknown)] 2.0}. All input values used for the modelling are compiled in Table 3. All included reactions, with corresponding dissociation constants, are given in the supplemental material (Section S2.4). The metal ion input concentrations for the predictions were based on the highest values of the measured concentrations of released Ni/Co from the stainless steels of this study. The input pH value for each fluid was based on the measured final pH value after exposure.

2.5. Surface analysis

Changes in oxidized metal composition of the outermost surface oxide (approx. 5–10 nm) of the stainless steel grades (304, 316 L, 430 and LDX2101) and the low-alloyed steel on surfaces unexposed and exposed (168 h) to the three synthetic fluids were evaluated by means of X-ray photoelectron spectroscopy (XPS, UltraDLD spectrometer, Kratos Analytical) using a monochromatic Al X-ray source (150 W) on areas approximately sized 700 × 300 μm^2 . Wide and detailed scans (pass energy: 20 eV) of the main elements, Cr 2p, Mn 2p, Ni 2p, Fe 2p and O 1s, were acquired and corrected to the C 1s contamination peak (285.0 eV). Since no Mn was observed for any of the stainless steels, the overlap between the Ni-LMM Auger and Mn 2p peaks was not considered. The background was subtracted using linear baselines. Deconvolution of peaks was based on their metallic and oxidic peak positions (peak positions given in Section 3.1.).

Table 3

Input settings for JESS modelling in three different fluids. ASW: 30 °C, pH 5–7, and pe 5 ($E_h = 308 \text{ mV}$); ASL: 37 °C, pH 6.75, and pe 5; GST: 37 °C, pH 1.5, and pe 5. The solvent was water (H₂O), and the metal input concentrations were 100 $\mu\text{g}/\text{L}$ for Ni (Ni²⁺) and 10 $\mu\text{g}/\text{L}$ for Co (Co²⁺), based on the highest measured released concentrations of Ni and Co from the stainless steel coupons in each fluid. Calculations were performed at the calculated equilibrium ionic strength and at 1.013 bar in all cases.

| Species (JESS input base species) | Species | ASW (M) | ASL (M) | GST (M) |
|-----------------------------------|--|---------|----------|---------|
| Na + 1 | Na ⁺ | 0.086 | 0.012514 | – |
| Cl-1 | Cl ⁻ | 0.086 | 0.0228 | 0.027 |
| K + 1 | K ⁺ | – | 0.016 | – |
| Urea | (NH ₂) ₂ CO | 0.017 | 0.017 | – |
| Lactic-1 | CH ₃ CHOHCO ₂ ⁻ | 0.011 | – | – |
| PO4-3 | PO ₄ ³⁻ | – | 0.0057 | – |
| H + 1(2)S-2 | H ₂ S | – | 6.94E-06 | – |

2.6. Electrochemical measurements

Differences in corrosion resistance of the stainless steel grades (304, 316 L, 430 and LDX2101) and the low-alloyed steel in the same fluids as for the metal release studies were determined by means of potentiodynamic polarization and electrochemical impedance spectroscopy (EIS) performed using a PARSTAT MC Multichannel Potentiostat (Princeton Applied Research) equipped with the VersaStudio software. Measurements were performed using a three-electrode electrochemical cell with the alloys/metals as the working electrode, an Ag/AgCl saturated KCl reference electrode, and a platinum mesh as a counter electrode. Prior to the measurements, all coupons were prepared as described in Section 2.1.

The polarization measurements were carried out in the most acidic fluid GST (pH 1.5) for all alloys, and additionally in all three fluids for the stainless steel grade 316 L. After the open circuit potential (OCP) stabilized for 1 h, the potential was swept from -0.2 V to 1 V vs. OCP at a scan rate of 1 mV/s. After the measurements, the coupons were rinsed with ultrapure water, dried with nitrogen gas, and placed in a desiccator at room temperature, prior to their surface observation by means of light optical microscopy (LOM, Leica DM2700 M) and scanning electron microscopy (SEM, tabletop TM-1000 Hitachi microscope).

The corrosion potential (E_{corr}) and corrosion current (I_{corr}), together with cathodic (β_c) and anodic (β_a) Tafel constants, were obtained by Tafel fitting of the polarization curve using the VersaStudio software. The corrosion current density (i_{corr}) was calculated from I_{corr} normalized to the exposed surface area (0.79 cm^2). R_p , the polarization resistance, was calculated from Eq. (2).

$$R_p = \frac{\beta_c \cdot \beta_a}{2.3 \cdot (\beta_c + \beta_a) \cdot i_{\text{corr}}} \quad (2)$$

EIS was performed in GST at OCP, after 1 h stabilization, by applying an alternating current (AC) voltage with an amplitude of 10 mV_{rms} and sweeping the frequencies from 10,000 Hz to 0.01 Hz. Details on the analysis of the data, based on average data from 4 to 5 measurements, are given in Section S1.2 in the supplemental material.

2.7. Relative bioaccessibility and bioaccessible concentrations

The relative bioaccessibility (the released amount of Ni or Co normalized to the bulk alloy content compared to the released amounts of Ni or Co from Ni or Co metal) was calculated from Eq. (3),

$$\text{Relative bioaccessibility}_{\text{Ni or Co}} = \frac{\left(\text{Released amount}_{\text{alloy}} \left(\frac{\mu\text{g}}{\text{cm}^2} \right) \right) \cdot \text{Bulk}_{\text{metal}} (\text{wt}\%)}{\left(\text{Released amount}_{\text{metal}} \left(\frac{\mu\text{g}}{\text{cm}^2} \right) \right) \cdot \text{Bulk}_{\text{alloy}} (\text{wt}\%)} \quad (3)$$

where the released amount_{alloy/metal} is the amount of Ni or Co released per surface area from the alloy (stainless steels or low-alloyed steel) or the metal, and the bulk_{alloy/metal} is the bulk content of Ni or Co.

The bioaccessible concentration, based on a relative comparison of the released amount per surface area from the alloy with the released amount from the metal, was calculated using Eq. (4).

$$\text{Bioaccessible concentration (wt.}\%) = \frac{\left(\text{Released amount}_{\text{alloy}} \left(\frac{\mu\text{g}}{\text{cm}^2} \right) \right)}{\left(\text{Released amount}_{\text{metal}} \left(\frac{\mu\text{g}}{\text{cm}^2} \right) \right)} \cdot 100 \text{ wt.}\% \quad (4)$$

2.8. Statistical evaluation

Kaleidagraph (Synergy, v. 4.0) was used to calculate a student's *t*-test (unpaired data, unequal variance) to compare two sets of

independent data statistically. A *p*-value of less than 0.05 was counted as a statistically significant difference.

3. Results and discussion

3.1. Environment-induced change in surface oxides

Fig. 1 shows that the surface oxide of all stainless steels, investigated by means of XPS, was composed of Fe(II)/Fe(III) (709.3 ± 0.6 eV, 711.0 ± 0.5 eV, 713.4 ± 0.6 eV) and Cr(III) (576.5 ± 0.3 eV, 578.0 ± 0.3 eV) oxides [29]. Only oxidized Fe (708.7 ± 0.4 eV, 710.8 ± 0.2 eV, 713.3 ± 0.3 eV, possibly attributed to FeO), but no Cr oxides, were observed on the surface of the low-alloyed (0.017 wt% Cr) steel. No Ni oxides were identified in the outermost surface layer of any of the investigated alloys. For 316 L and 304, Ni was only observed in its metallic form (Ni 2p: 852.9 ± 0.1 eV), which is related to its presence beneath the surface oxide [30,31]. Oxidized Cr was observed in its trivalent form, providing good corrosion resistance to the stainless steels [32]. A large increase (3–4-fold, $p < 0.05$) of the oxidized Cr content after 168 h of exposure compared with the oxidized Cr content under unexposed conditions was evident in GST (pH 1.5) for all stainless steel grades, while there were only slight changes after exposure to ASW ($p > 0.05$), and a reduced Cr content after exposure to ASL ($p < 0.05$ for 316 L and LDX2101, $p > 0.05$ for 304 and 430). These results are further discussed below. The enrichment of Cr within the outermost surface at acidic conditions is in line with previous findings [33].

The thickness of the surface oxide of stainless steels increased after exposure to ASL ($p < 0.001$), as judged semi-quantitatively from the increasing relative mass ratio of oxidized to non-oxidized metal peaks, Table S1 (supplemental material). No statistically significant changes in this ratio were found for the other two solutions as compared to unexposed conditions for the stainless steels.

3.2. Corrosion resistance

Potentiodynamic polarization curves of stainless steel grade 316 L in all fluids are presented in Fig. 2a and for all alloys (stainless and low-alloyed steel) in the most acidic solution (GST) in Fig. 2b, together with their corresponding post-polarization LOM images. Corresponding corrosion parameters (corrosion potential, corrosion current density, polarization resistance, and pitting potential) of each alloy evaluated from the polarization curves are summarized in Table 4.

The corrosion potential, E_{corr} , decreased for 316 L according to ASW > ASL > GST ($p < 0.05$ when comparing ASW with ASL and GST), Table 4, but no clear trend was observed for the polarization

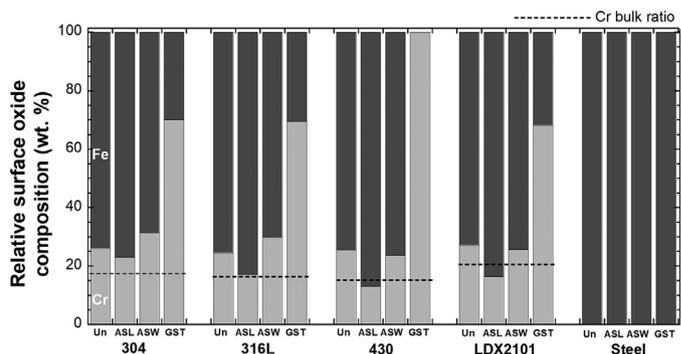


Fig. 1. Relative mass content (wt%) of oxidized metals (Fe and Cr) in the outermost surface oxide of the stainless steels (304, 316 L, 430, and LDX2101) and the low-alloyed steel measured by means of XPS, prior to (unexposed – Un) and after exposure to ASL (pH 6.75), ASW (pH 6.5) and GST (pH 1.5) for one week. Average values of duplicate independent coupons (each measured twice at different surface locations) with standard deviations given in Table S1 (supplemental material).

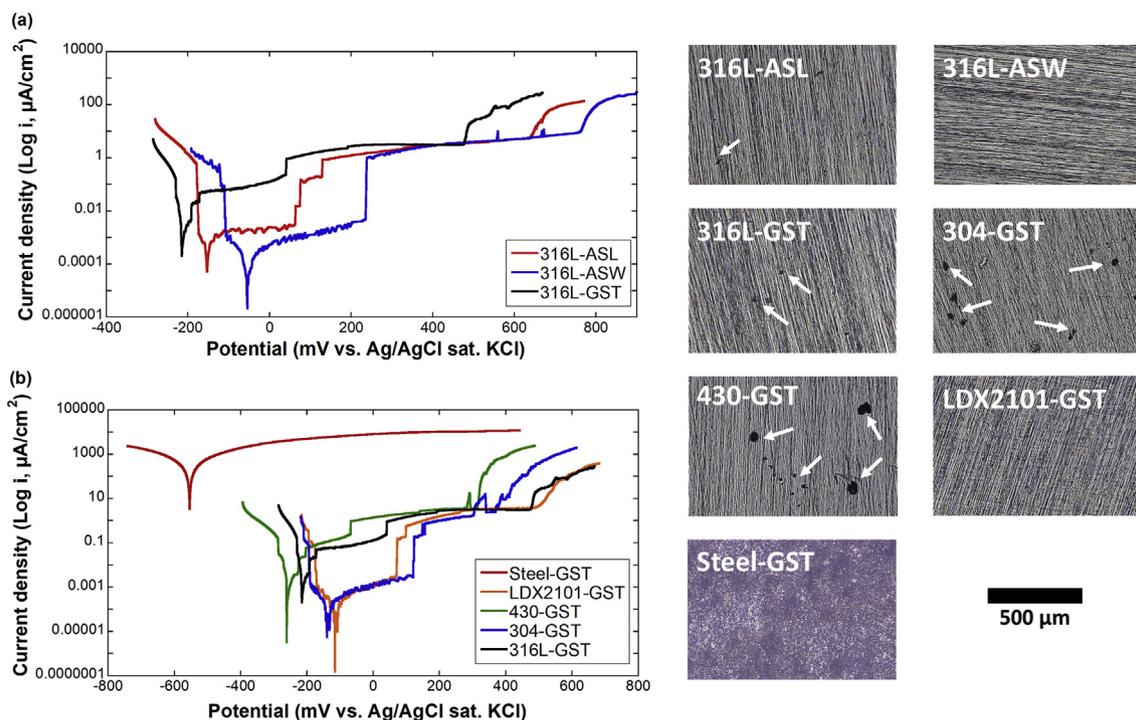


Fig. 2. Representative current density – potential curves of (a) stainless steel grade 316 L exposed to ASL (pH 6.75), ASW (pH 6.5) and GST (pH 1.5); (b) 316 L, 430, 304, LDX2101, and low-alloyed steel exposed to GST. Information on measurement variability can be found in Table 4. Corresponding post-polarization LOM images are included, and the arrows highlight the presence of pits. Detailed SEM images of pits are shown in Fig. S3 (supplemental material).

resistance (R_p), corrosion current density (i_{corr}), or the pitting potential (E_{pp}), under similar conditions. Very few pits were observed on any of the triplicate 316 L coupons in ASW (Fig. 2 and Fig. S3, supplemental material). In both ASL and GST, one of two 316 L coupons showed evident pit formation all over the surface after the potentiodynamic polarization observed in both LOM and SEM images (Figs. S2–S3, supplemental material), while the other coupon only showed a few pits. Exposure of 316 L to ASL seemed to result in more severe corrosion than exposure to ASW, as judged from differences observed in the LOM and SEM images and from slight differences in corrosion current density ($p > 0.05$). This could possibly be related to the high sulfide content of ASL [34]. The sulfide content of ASL may furthermore explain the reduced amount of Cr observed by means of XPS within the outermost surface oxide of all stainless steels exposed to ASL. These observations agree with previous findings on 316 L [34]. GST (diluted HCl) was clearly shown to be the most corrosive solution to the alloys. Diluted HCl is known to interact with the passive surface oxide on stainless steel, partially by reductive dissolution [26,35]. No statistically significant differences in polarization characteristics (E_{corr} , i_{corr} , R_p , and E_{pp}) were observed between the four different stainless steel grades in GST, Fig. 2b and Table 4. However, grades 430 and 304 immersed in GST were more susceptible to pitting corrosion, showing larger and

more discernible pits after polarization, Fig. 2 and Fig. S3 (supplemental material), with significantly lower E_{corr} ($p < 0.05$) and slightly higher i_{corr} on grade 430 than on 304. This is in line with the EIS results, Fig. S4 and Table S2, with the lowest charge transfer resistance ($113 \text{ k}\Omega \text{ cm}^2$) for 430 in GST as compared to $220\text{--}424 \text{ k}\Omega \text{ cm}^2$ for the other stainless steel grades. The low-alloyed steel corroded actively and was almost fully covered by corrosion products after the potentiodynamic scan, with a lower E_{corr} ($p < 0.01$), a lower R_p (up to 330,000-fold), and a higher i_{corr} (up to 580,000-fold) than observed for the stainless steel grades. The low-alloyed steel also showed a significantly lower charge transfer resistance in GST ($0.070 \text{ k}\Omega \text{ cm}^2$) than did the stainless steels ($113\text{--}424 \text{ k}\Omega \text{ cm}^2$), Fig. S4 and Table S2.

3.3. Ni and Co release from alloys and pure metals in synthetic biological fluids

3.3.1. JESS modelling

Table 5 shows the results of thermodynamic chemical speciation modelling. The modelling predicted Ni and Co to be totally dissolved (aqueous species) in GST (pH 1.5). Since ASW has a relatively low buffer capacity, its pH changed during exposure of different materials, and varied between 5 and 7 after exposure. JESS predicted Ni to be soluble over

Table 4

Corrosion potential (E_{corr}), corrosion current density (i_{corr}), corrosion resistance (R_p) and pitting potential (E_{pp}) of 316 L (in ASL, ASW and GST), and 304, 430, LDX2101 and low-alloyed steel (all in GST). At least two independent coupons for each grade (mean values and standard deviations are shown).

| Grade | Solution | E_{corr} (mV) | i_{corr} ($\mu\text{A}/\text{cm}^2$) | R_p ($\text{k}\Omega \cdot \text{cm}^2$) | E_{pp} (mV) |
|-------------------|----------|-----------------|--|--|---------------------|
| 316 L | ASL | -147 ± 13 | 0.00660 ± 0.0076 | 2350 ± 2000 | 534 ± 140 |
| | ASW | -53.0 ± 10 | 0.000743 ± 0.0010 | $71,000 \pm 60,000$ | 595 ± 140 |
| | GST | -196 ± 20 | 0.0198 ± 0.027 | 6000 ± 7900 | 436 ± 50 |
| 304 | | -128 ± 16 | 0.000975 ± 0.00088 | $33,100 \pm 42,000$ | 368 ± 11 |
| 430 | | -265 ± 18 | 0.061 ± 0.033 | 413 ± 220 | 281 ± 45 |
| LDX2101 | | -119 ± 5.0 | 0.0151 ± 0.021 | $37,500 \pm 52,000$ | 483 ± 16 |
| Low alloyed steel | | -545 ± 13 | 567 ± 168 | 0.116 ± 0.035 | Active ^a |

^a Active corrosion throughout the anodic polarization range.

Table 5Predominant species ($\geq 1\%$ predominance) as calculated by JESS for two metal ions (Ni and Co) and the three synthetic fluids (for input values see Table 3).

| Conditions | | Ni (100 $\mu\text{g/L}$) | Co (10 $\mu\text{g/L}$) |
|------------|---------|---|---|
| ASW | pH 5 | 88% Ni^{2+} (aq), 11% Ni-Cl^+ (aq), 1% NiCl_2 (aq) | 87% Co^{2+} (aq), 13% Co-Cl^+ (aq) |
| | pH 5.5 | 88% Ni^{2+} (aq), 11% Ni-Cl^+ (aq), 1% NiCl_2 (aq) | 87% Co^{2+} (aq), 13% Co-Cl^+ (aq) |
| | pH 6 | 88% Ni^{2+} (aq), 11% Ni-Cl^+ (aq), 1% NiCl_2 (aq) | 87% Co^{2+} (aq), 13% Co-Cl^+ (aq) |
| | pH 6.5 | 87% Ni^{2+} (aq), 11% Ni-Cl^+ (aq), 1% NiCl_2 (aq), 1% Ni-Lactic^+ (aq) | 86% Co^{2+} (aq), 13% Co-Cl^+ (aq), 1% Co-Lactic^+ (aq) |
| | pH 7 | 82% Ni^{2+} (aq), 10% Ni-Cl^+ (aq), 6% Ni-Lactic^+ (aq), 1% NiCl_2 (aq) | 91% Co_3O_4 (s), 7% Co^{2+} (aq), 1% Co-Cl^+ (aq), 1% Co-Lactic^+ (aq) |
| ASL | pH 6.75 | 100% $\gamma\text{-NiS}$ (s) | 100% $\beta\text{-CoS}$ (s) |
| GST | pH 1.5 | 94% Ni^{2+} (aq), 5% Ni-Cl^+ (aq) | 93% Co^{2+} (aq), 7% Co-Cl^+ (aq) |

aq – aqueous (dissolved), s – solid (precipitation possible), lactic – lactate ion (valency: -1).

this entire pH range. Co solubility was however predicted to decrease rapidly with increasing pH in ASW, from 100% dissolved at pH 5 to 9% dissolved at pH 7, Table 5. Neither Ni nor Co were predicted to be soluble in ASL (pH 6.75), as they form solid NiS or CoS, Table 5.

3.3.2. Ni release

Fig. 3 shows the amount of released Ni per unit surface area (Fig. 3a) and corresponding release rates (amount per unit surface area and hour) (Fig. 3b) for grade 316 L exposed to the different synthetic body fluids for 2, 4, 8, 24 and 168 h (independent coupons for each fluid and period). The release of Ni was highly pH/fluid- and time-dependent, Fig. 3a. In agreement with the corrosion resistance measurements, the release of Ni increased with increasing acidity of the test fluids (Fig. 2a). All release rates strongly decreased with increasing exposure time. The highest amount of Ni was released in the most acidic fluid, GST (pH 1.5), for all exposure periods (reaching $0.074 \pm 0.0026 \mu\text{g}/\text{cm}^2$ after 168 h of exposure). No evident increase in Ni release was observed after 8 h in GST, but a substantial increase was observed after 24 h, possibly indicative of a less passive surface oxide. The amount of released Ni was seemingly reduced with exposure time after 2 h in ASL (pH 6.75), findings consistent with chemical speciation modelling results (Table 5) that predicted Ni to form solid NiS in ALS (no free or labile ions). In ASW, the release rates of Ni first increased up to 8 h before declining up to 168 h (Fig. 3b). Such a release behaviour has previously been associated with a delayed complexation-induced metal release mechanism [36]. Ni ion complexation in the ASW fluid was supported by the speciation modelling results, which predicted the formation of Ni-lactate and Ni-Cl complexes, Table 5.

When comparing the released amounts of metals from the different grades and metals after 4 and 168 h, we see that the rates were, in most cases, greater after 168 h than at 4 h, but not proportionally greater, as expected from the longer time period (42-fold longer), Fig. 4. This means that, in all cases, some extent of surface passivation, mass transport limitation, or solution saturation effects took place with time, which reduced the release rate. Compared to Ni metal, the stainless

steel grades (304, 316 L, 430 and LDX2101) released very low amounts (up to 80,000-fold lower after 4 h, and up to 300,000-fold lower after 168 h) of Ni into all fluids. Despite a higher Ni bulk content, the stainless steels released similar amounts of Ni compared with the low-alloyed steel after 4 h and lower amounts (up to 400-fold, $p > 0.05$) after 168 h, which is explained by the less protective surface oxide (Fig. 1) and lower corrosion resistance (Fig. 2b) of the low-alloyed steel after immersion into the synthetic fluids. The ferritic (430) and duplex (LDX2101) grades released very low amounts of Ni ($< 0.01 \mu\text{g}/\text{cm}^2/\text{week}$) into all fluids, probably related to their low bulk Ni content, and also high corrosion resistance for LDX2101, in agreement with previous findings for the duplex grade LDX2205 [37].

3.3.3. Co release

Fig. 5 shows the released amount of Co (Fig. 5a) and corresponding release rates (Fig. 5b) for grade 316 L in the different synthetic fluids. Co was released to a lower extent than Ni (Fig. 3), primarily due to its substantially lower bulk content (0.24 wt% Co, 10 wt% Ni). The highest released amount of Co was observed in the most acidic fluid (GST), followed by ASW. Chemical speciation modelling predicted released Co to precipitate in both ASL (as solid CoS) and ASW (as solid Co_3O_4), Table 5. This could explain the very low levels of Co in solution observed in ASL and the reduced (not statistically significant) amounts of released Co after 168 h compared with 24 h in ASW, Fig. 5. The formation of Co-lactate and Co-Cl complexes predicted to take place in ASW, Table 5, could possibly explain the initial increased amounts of released Co in ASW up to 24 h, related to a delayed complexation-induced release process.

Compared with the release of Co from Co metal, the release of Co was substantially lower (up to 1,600,000-fold, $p < 0.01$ in GST, $p < 0.05$ in ASW, and $p > 0.05$ in ASL) from all stainless steel grades, while differences between the stainless steel grades were small (Fig. 6). The low-alloyed steel, which contains only 0.015 wt% Co, released more Co (up to 300-fold) into all fluids than did the stainless steels with bulk contents of 0.019–0.24 wt% Co. Almost negligible

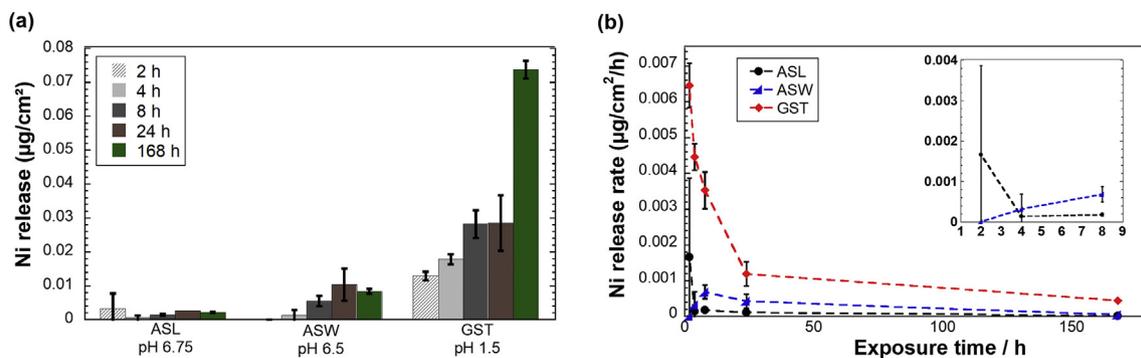


Fig. 3. Released amounts of Ni per unit surface area ($\mu\text{g}/\text{cm}^2$) (a) and corresponding release rates ($\mu\text{g}/\text{cm}^2/\text{h}$) (b) for stainless steel 316 L exposed to ASL (pH 6.75), ASW (pH 6.5) and GST (pH 1.5) for 2, 4, 8, 24 and 168 h (1 week). The error bars represent the standard deviation of triplicate coupons. The inset graph in (b) magnifies the initial (first 8 h) release rates of Ni from 316 L in ASL and ASW.

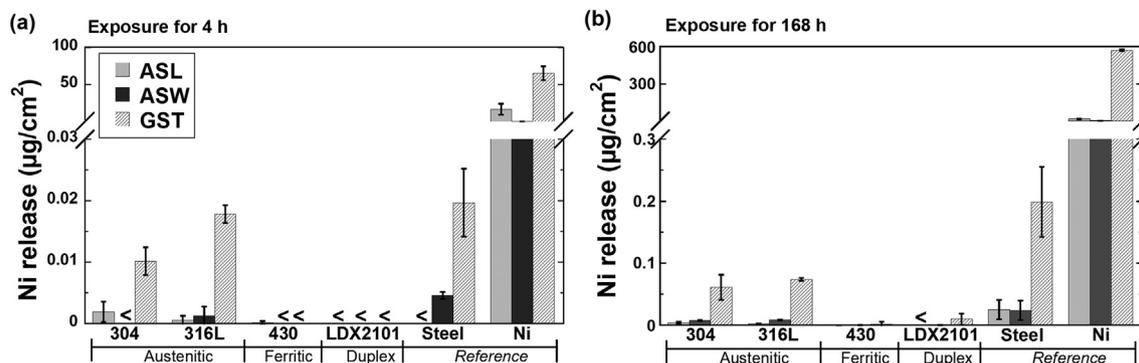


Fig. 4. Released amount of Ni per unit surface area ($\mu\text{g}/\text{cm}^2$) from different stainless steels (austenitic- 304 and 316 L, ferritic - 430, and duplex- LDX2101) immersed in ASL (pH 6.75), ASW (pH 6.5) and GST (pH 1.5) for 4 h (a) and 168 h (b). Data on the low-alloyed steel and Ni metal are included for comparison. Note the different scales on the ordinate axis in a) and b). The error bars represent the standard deviation of triplicate coupons. < < denotes below limit of detection.

amounts of released Co were observed for LDX2101 in all fluids after long-term exposure (168 h), findings consistent with its release pattern of Ni and corrosion resistance performance in GST (Fig. 2b, Table 4, Fig. S4 and Table S2).

3.4. Relative bioaccessibility

3.4.1. Relative bioaccessibility and bioaccessible concentration of Ni

Fig. 7 shows the relative bioaccessibility [calculated from Eq. (3)] of released Ni from the alloys after 4 and 168 h exposure in the different fluids. Results of Ni metal are included for comparison and equal 1 per definition. A relative bioaccessibility of 1 means that Ni in the alloy behaves similar to Ni metal from a metal release perspective. All stainless steel grades showed a relative bioaccessibility of Ni substantially less than 1 (ranging from 0.00033 to 0.039, that is, 25- to 3000-fold lower than expected from the bulk content) in all fluids. This means that they release much less Ni than would be expected from their bulk Ni content in the synthetic body fluids investigated. This is probably a result of their high corrosion resistance compared to that of Ni metal. The relative bioaccessibility of Ni for the low-alloyed steel was, in contrast, substantially higher than or equal to (up to 32-fold higher than expected from the bulk content) that of the Ni metal, which is expected for materials of lower corrosion resistance than Ni metal.

From the calculated bioaccessible concentrations [from Eq. (4)] of Ni, compiled in Table S3 (supplemental material), it is evident that all stainless steels in this study behave like alloys containing 0.00034–0.3 wt% Ni rather than 0.11–10 wt% Ni as being their bulk content, i.e. a considerable positive alloying effect. In the case of

the low-alloyed steel, the calculated bioaccessible concentration (0.030–1.1 wt% Ni) was similar to or greater than the corresponding bulk content (0.034 wt% Ni).

3.4.2. Relative bioaccessibility and bioaccessible concentration of Co

The relative bioaccessibility and the bioaccessible concentrations of Co released from all alloys in all fluids after 4 and 168 h of exposure are presented in Fig. 8 and Table S4, respectively. Consistent with Ni findings, there is a distinct positive alloying effect on Co in stainless steels (a relative bioaccessibility less than 1 for released Co, ranging from 0.00098 to 0.71) and a negative alloying effect for the low-alloyed steel (a relative bioaccessibility exceeding 1, ranging from 1.1 to 59). This means less release of Co (2–1000-fold) from the stainless steels than what would be expected from their respective bulk alloy contents. Due to time-dependent precipitation of released Co in solution, primarily in ASL, Table 5, the relative bioaccessibility increased for most grades, due to a more significant (10-fold, $p < 0.001$) reduction of released Co in solution from Co metal with time (4 h - $10 \mu\text{g}/\text{cm}^2$; 168 h - $1 \mu\text{g}/\text{cm}^2$). The faster precipitation of Co from ASL solution for the high-releasing Co metal than for the alloys is a result of the high solution concentration of Co, an increased solution pH due to the on-going corrosion reactions, and the thermodynamic instability of aqueous Co in ASL (c.f. Section 3.3.1.). Less Co than expected from the bulk alloy content (0.019–0.24 wt% Co) was released from the stainless steels, and their bioaccessible concentrations of Co were determined to vary between 0.000061 and 0.11 wt%. The bioaccessible concentration of released Co from the low-alloyed steel (0.017–0.88 wt% Co) exceeded its bulk content (0.015 wt% Co) for all conditions.

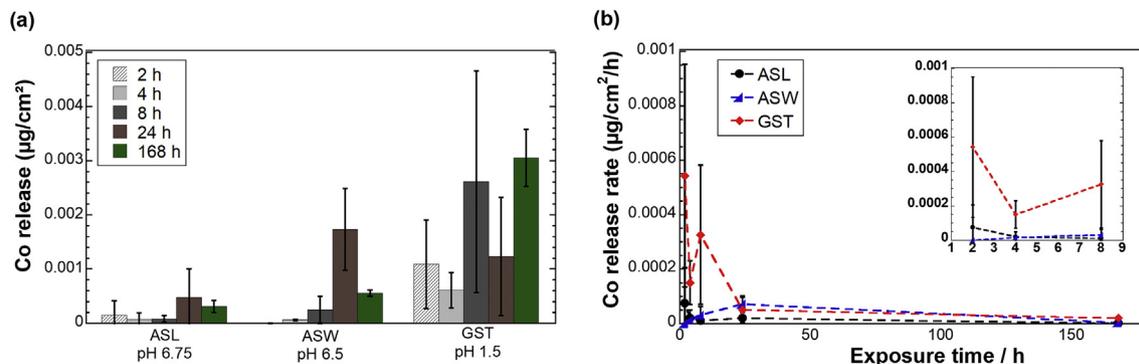


Fig. 5. Released amounts of Co per unit surface area ($\mu\text{g}/\text{cm}^2$) (a) and corresponding release rates ($\mu\text{g}/\text{cm}^2/\text{h}$) (b) for stainless steel 316 L exposed to ASL, ASW and GST for 2, 4, 8, 24 and

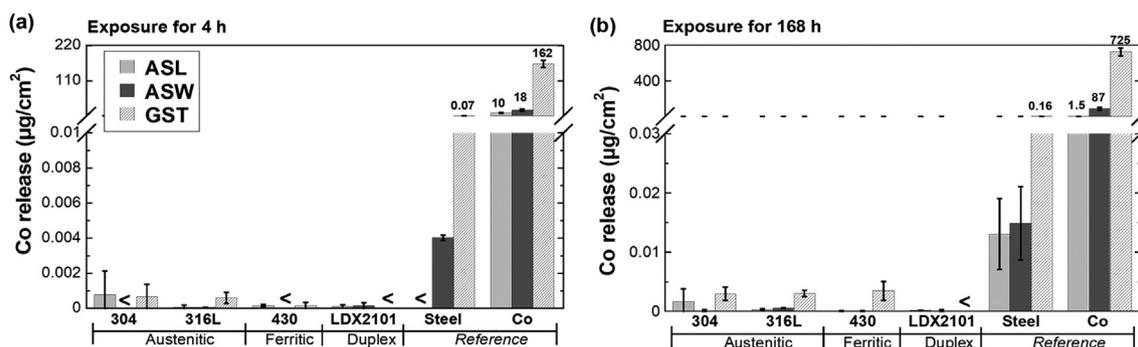


Fig. 6. Released amount of Co per unit surface area ($\mu\text{g}/\text{cm}^2$) from different stainless steels (austenitic– 304 and 316 L, ferritic – 430, and duplex– LDX2101) immersed in ASL (pH 6.75), ASW (pH 6.5) and GST (pH 1.5) for 4 h (a) and 168 h (b), respectively. Data on the low-alloyed steel and Co metal are included for comparison. The error bars represent the standard deviation of triplicate coupons, and < denotes below limit of detection.

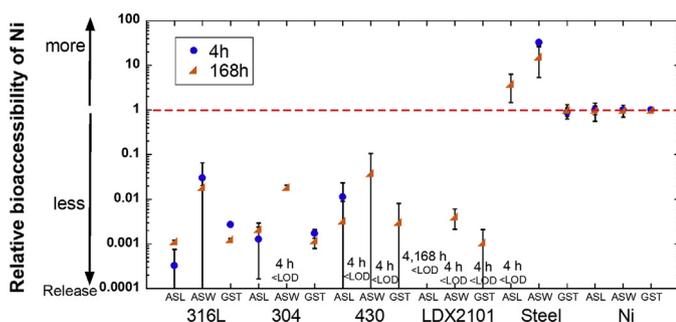


Fig. 7. Relative bioaccessibility of Ni (logarithmic scale) determined for all alloys exposed to ASL, ASW and GST for 4 and 168 h. The relative bioaccessibility of Ni metal equals 1 per definition, Eq. (3), which is marked as a dotted line. <LOD means below limit of detection.

3.5. Implications on hazard assessment of alloys

The relative bioaccessibility determined for Ni and Co in this study differed greatly among the investigated alloys, being substantially lower than 1 for the stainless steels and mostly higher than 1 for the low-alloyed steel. These findings are related to differences in the surface characteristics and corrosion resistance of the alloys. Stainless steels have a higher Ni and Co bulk content than the low-alloyed steel, but show lower bioaccessibility of both Ni and Co. The low-alloyed steel of low corrosion resistance released more Ni and Co than expected from its bulk content. Observed findings suggest that the bioaccessible concentration, rather than the bulk metal content, should be used for hazard assessment of alloys.

A positive example of considering chemical and material properties in hazard assessment is the Nickel Directive [38], which stipulates a restriction limit of Ni for items intended to come into skin contact, based

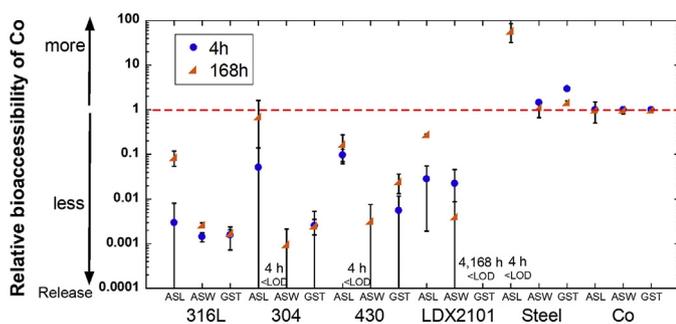


Fig. 8. Relative bioaccessibility of Co (logarithmic scale) determined for all alloys exposed into ASL, ASW and GST for 4 and 168 h. The relative bioaccessibility of the Co metal equals 1 per definition, Eq. (3), which is marked as a dotted line. <LOD means below limit of detection.

on Ni release data from bioelution testing. However, both Co and Ni classification for other human exposure routes and applications are, so far, based on the bulk alloy content.

In this study, all alloys released very low amounts of Ni into ASW, less than $0.2 \mu\text{g Ni}/\text{cm}^2/\text{week}$, a restriction limit set for items to be inserted into pierced body parts [38]. According to recent literature [39], skin doses eliciting contact dermatitis in 10% of Co-allergic individuals (ED10) and in 50% of Co-allergic individuals (ED50) were reported in the ranges of $0.066\text{--}1.95 \mu\text{g}/\text{cm}^2$ and $1.45\text{--}17 \mu\text{g}/\text{cm}^2$, respectively. Although these limits are derived from fully soluble metal salts (not the metal) applied for 48 h to the skin and normalized to the exposed skin area (not surface area of the metal), they can, to some extent, be compared with findings of this study. All alloys in this study released less than $0.015 \mu\text{g Co}/\text{cm}^2$ in ASW for all time points up to one week, which is substantially lower than the lowest reported ED10.

The maximum allowed concentration of Ni in drinking water is $70 \mu\text{g}/\text{L}$ [40], while at most $140 \mu\text{g Ni}/\text{L}$ food is stipulated for protection of Ni-sensitised individuals [41]. It should be acknowledged that any direct comparison of concentrations can be misleading as they are highly surface area to solution volume dependent. The surface area to solution volume ratio of this study was approximately $1 \text{ cm}^2/\text{mL}$, which is a high ratio considering relevant food contact [42] and resulted, hence, in relatively high concentrations. Despite this high ratio, all alloys investigated in this study released significantly less Ni in the simulated oral route - ASL (highest concentrations released from grade 304 ($3.47 \mu\text{g}/\text{L}$) compared with $25.2 \mu\text{g}/\text{L}$ from the low-alloyed steel). In GST (ingestion), the highest concentration of Ni released from the stainless steels was observed for grade 316 L ($78.4 \mu\text{g}/\text{L}$) compared with $214.5 \mu\text{g Ni}/\text{L}$ for the low-alloyed steel.

The corresponding limit of Co in drinking water is $20 \mu\text{g}/\text{L}$ [40]. In this study, the amount of Co release from stainless steels per unit volume was at most $4 \mu\text{g}/\text{L}$ in ASL and GST, while the Co release from the low-alloyed steel was higher ($12.9 \mu\text{g}/\text{L}$ in ASL and $174 \mu\text{g}/\text{L}$ in GST). These comparisons elucidate that prevailing surface and corrosion properties of the alloys govern the release of bioaccessible metals, effects that are not possible to predict from the bulk alloy composition. These aspects are already reflected in their wide use in applications, but not yet implemented in chemical legislation.

4. Conclusions

- Only small changes in the oxidized Cr(III) to Fe(II/III) mass ratio of the outermost surface oxide were observed for any of the stainless steel grades (austenitic – AISI 304, 316 L; ferritic – AISI 430; duplex – LDX2101) after exposure to ASW and ASL. Exposure to the most acidic fluid, GST, resulted in a strongly increased Cr surface content. The surface oxide of the low-alloyed steel contained only Fe-oxides without any evident compositional changes upon fluid exposure.

- A higher corrosion resistance was observed on all stainless steels than on the low-alloyed steel upon exposure to GST.
- The stainless steel grade 316 L showed Ni and Co release rates that strongly decreased with time in all solutions, especially in GST.
- All stainless steel grades released very low amounts of Ni into all solutions (up to 400-fold lower after 168 h than the low-alloyed steel), and also substantially lower amounts of Ni (up to 300,000-fold after 168 h) than Ni metal. The extent of Ni release increased with the acidity of the test fluids. In contrast with the release pattern of the low-alloyed steel, the release of Ni from all stainless steels was 25 to 3000-fold lower than expected based on its bulk contents. The stainless steels behaved like alloys containing 0.00034–0.3 wt% Ni rather than 0.11–10 wt% Ni as in their actual bulk content, elucidating a positive alloying effect. As a result of the low corrosion resistance of the low-alloyed steel (negative alloying effect), it behaved like an alloy containing 0.030–1.1 wt% Ni, i.e., findings similar to or greater than its bulk content (0.034 wt% Ni).
- The amount of released Co increased with the acidity of the synthetic biofluid. The bioaccessible concentrations of Co (0.000061–0.11 wt%) were lower for the stainless steels than their bulk contents (0.019–0.24 wt% Co). The bioaccessible concentration of the low-alloyed steel (0.017–0.88 wt% Co) exceeded its bulk content (0.015 wt% Co) for all exposure conditions.
- The duplex stainless steel, LDX2101, released, for most exposure conditions and fluids, the lowest amounts of both Ni and Co, findings in agreement with the highest corrosion resistance among the investigated stainless steel grades.
- Bioaccessibility data rather than relative bulk alloy contents should be used to refine current hazard assessments and classifications of alloys within regulations in order to more realistically assess the behaviour of alloys under certain exposure conditions, and thereby elucidate both positive and negative alloying effects.

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Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to legal or ethical reasons.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.matdes.2020.109393>.

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