Attachment 20

Summaries of ECTX Gastric Bioelution Studies

Study 1 (2017). To demonstrate how the **presence of a matrix effect in alloys can affect metal releases** compared to those from simple mixtures, the following experiment was carried out by ECTX (2017). The release of nickel from a sample of SS316L alloy powder containing 10.7% of Ni (68.6% Fe, 16.4% Cr) was compared to the release of nickel from a simple mixture containing 10.6% Ni, 68.6% Fe, and 16.4% Cr powders, both samples tested at the same sample loading of 2 g/L. Triplicate test samples with duplicate sampling per vessel were included. Gastric fluid corresponded to an HCl solution at pH 1.5 and the duration of the test was 2 hours. The release from pure Ni metal powder tested at a 0.2 g/L loading was also reported. The results shown in Table 1 below demonstrate a 300-fold difference between the Ni releases from a simple mixture compared to release from the previously tested SS316L alloy (ECTX 2016), even if the concentration of Ni in both samples was the same. Using the release of pure Ni metal as a reference, the bioaccessible concentrations (BC) of Ni in the simple mixture (12%) and SS316 L (0.04%) were calculated. The BC of Ni in the simple mixture (12%) coincides with its bulk concentration (11%) indicating that no matrix effect was present. By contrast, the BC of Ni in SS316L (0.04%) is ~300-fold lower than its bulk concentration (11%) and 300-fold lower than the BC of the simple mixture (12%). This illustrates how the alloy matrix effect can affect metal bioaccessibility.

Sample	***Particle diameter***	***Mass loading (g/L)***	***Nickel loading (g Ni/L)***	***Nickel release (mg Ni/g sample)***	***Bioaccessible Concentration of Ni in mixture (%)***
Simple mixture: *68.6% Fe, 16.4% Cr, 10.7% Ni*	D_{90}= 2 µm	2	0.21	20.0	12
SS316L: *68.6% Fe, 16.4% Cr, 10.7% Ni*	D_{80}= 5 µm	2	0.21	0.061	0.04
Ni metal, *100% Ni*	D_{90}= 2 µm	0.2	0.2	163	

Table 1. Differences in metal release in gastric fluid from a simple mixture and an alloy containing the same bulk concentration of nickel (ECTX 2017).

While the particle size of the powders tested above was quite similar (D90 =2-5 µm), the surface area of the Ni metal powder in the simple mixture (2.58 m^2/g) was different from that of the SS316L alloy powder (0.35 m^2/g). If desired, the bioaccessible concentrations can be re-calculated after applying a correction for measured surface area of the samples.

Study 2 (2017). In this study, **the effect of loading on metal release** in gastric fluid was examined. The metal releases from a simple mixture containing 68.6%Fe, 16.4%Cr and 10.7% Ni were measured in gastric fluid (pH 1.5) after 2 hours at 3 different loadings: 0.2, 1, and 2 g/L. Triplicate test samples with duplicate

sampling per test vessel were included. Single vessels with duplicate sampling per vessel were included for pure reference Fe, Cr and Ni metals. Results are shown in Table 2 and Figure 1 below.

Samples	**Metal releases after 2h in gastric fluid (µg metal/g sample g ± SD sample)**		
	0.2 g/L Loading	**1g/L Loading**	**2g/L Loading**
Simple Mixture			
Fe (68.6%)	318,986 ± 6,360	252,631 ± 7,191	218,725 ± 1,502
Cr (16.4%)	73 ± 6.5	30 ± 1	22 ± 0.2
Ni (10.7%)	25,226 ± 1,340	29,217 ± 1,090	20,073 ± 564
Pure metals			
Fe	420,925		
Cr	101		
Ni	162,734		

Table 2. Metal releases after 2h in gastric fluid from a simple mixture at 3 different loadings and pure ingredients at low loading (ECTX, 2017).

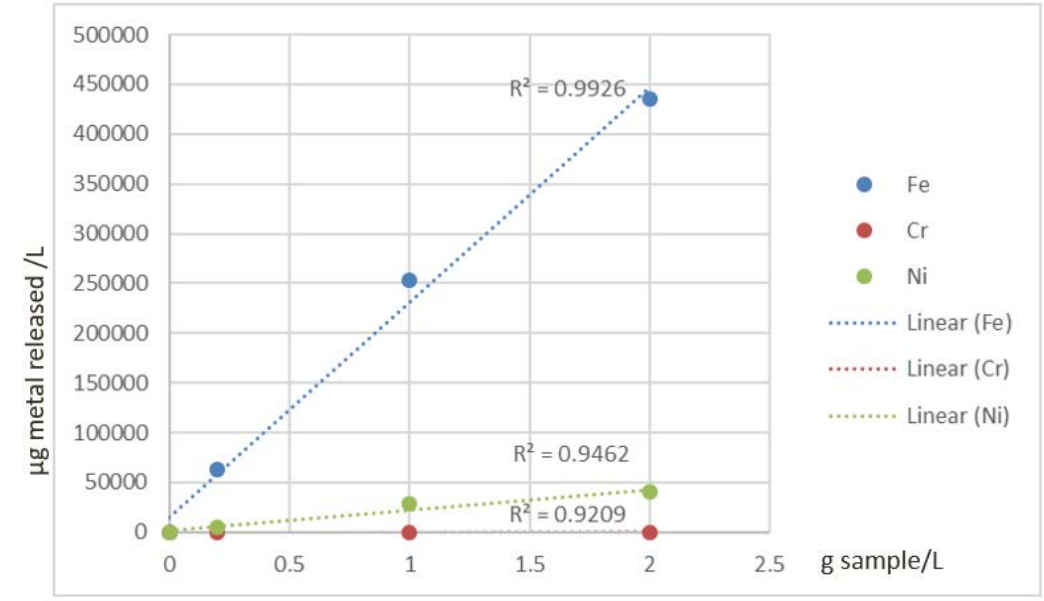


Figure 1: Effect of loading on metal release using a mixture of Fe, Cr and Ni in gastric fluid after 2 h and using 3 different loadings (ECTX 2017)

The results showed reasonably linear relationships between metal releases and loading for the 3 metals tested Figure 1). They also show that different metals have different bioaccessibilities in gastric fluid (e.g. Fe and Cr differ by more than 10-fold in metal release per gram of metal, Table 2). On a per gram of sample basis the releases decreased as the loading increased for Fe and Cr, while remaining the same for Ni Table 2).

Study 3 (2018a). A study was carried out to examine the **influence of sample mass, either as inert material (SiO_2) or as alloy material on the release of metals**. The study measured Ni release (after 2 hours in gastric fluid) from the following three samples: Ni metal powder (0.21 g/L sample loading), Ni metal powder (0.21 g/L loading) + SiO_2 (1.79 g/L loading) for a total sample loading of 2 g/L, and SS316L with a content of 10.7% Ni (2 g/L sample loading). Single test samples with duplicate samplings per test vessel were included for nickel and SS316L powder, while triplicate test samples (with duplicate samplings per test vessel) were included for the mixture of nickel metal and SiO_2. The results from this study are shown in Table 3 below.

Sample powders	*Mass loading (g/L)*	*Nickel loading (g Ni/L)*	*Nickel release (mg Ni/L)*	*Nickel release (mg Ni/g sample)*	*% Ni released*
Ni metal	0.21	0.21	44.6	212.6	21
Ni metal + SiO_2	2.0	0.21	53.0	26.5	25
SS316L	2.0	0.21	0.1	0.065	0.06

Table 3: Gastric bioelution results for Ni containing samples tested at equal mass or equal nickel loading (nickel measurements in blank and pure SiO2 samples were below the reporting limit of 0.5 µg/L) (ECTX 2018a).

Table 3 results confirm that the lower percent nickel release from SS316L powder compared to pure nickel metal powder of similar particle characteristics is not due to a mass effect (i.e., a higher mass loading used for alloy than for pure metal) but it is due to the matrix alloy effect. Furthermore, they indicate that the addition of 10-fold higher mass of substance does not affect the release of Ni from Ni metal powder to any significant extent. The results in Table 3 are comparable to those previously reported (Table 1).

Study 4 (2018b). In this study, the **kinetics of metal release in gastric fluid** were examined. The objective of the study was to assess if saturation of metal release from alloys and their pure metal ingredients could be observed at the time period of 2 hours which has been selected for the gastric fluid bioelution test. For this purpose, both powders and massive forms of alloys and ingredients were tested.

The following <u>powder</u> samples were tested: an alloy (SS316L), two of its pure ingredients (Fe^o and Ni^o) and two water soluble compounds of these metal ingredients ($FeSO_4$ $7H_2O$ and $NiCl_2$ $6H_2O$). Measurements were carried out after 1, 2 and 4 hours in gastric fluid (pH 1.5) at a loading of 2g/L. It was reasoned that if no saturation of metal release was observed at the higher loading of 2 g/L, no saturation would be expected to occur at the lower loading of 0.2 g/L. Single sample vessels were included at each time point, except for SS316L were duplicate samples were tested; each vessel was sampled twice. The results for the pure ingredients (Fe^o and Ni^o) and the two water soluble compounds ($FeSO_4$ $7H_2O$ and $NiCl_2$ $6H_2O$) are shown in Figure 2; the results for SS316L are shown in Figure 3.

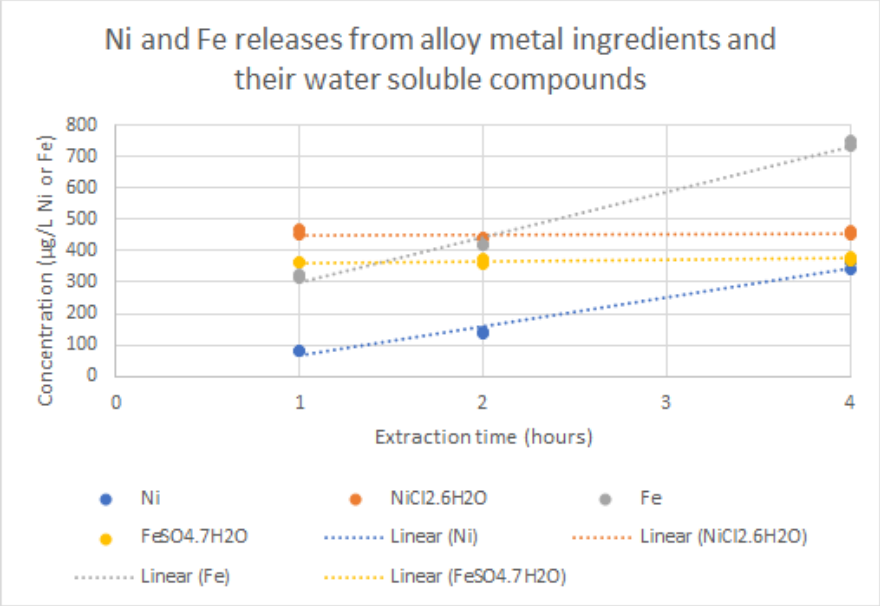


Figure 2: Time-dependent metal release in gastric fluid for pure metals and compounds (powder forms).

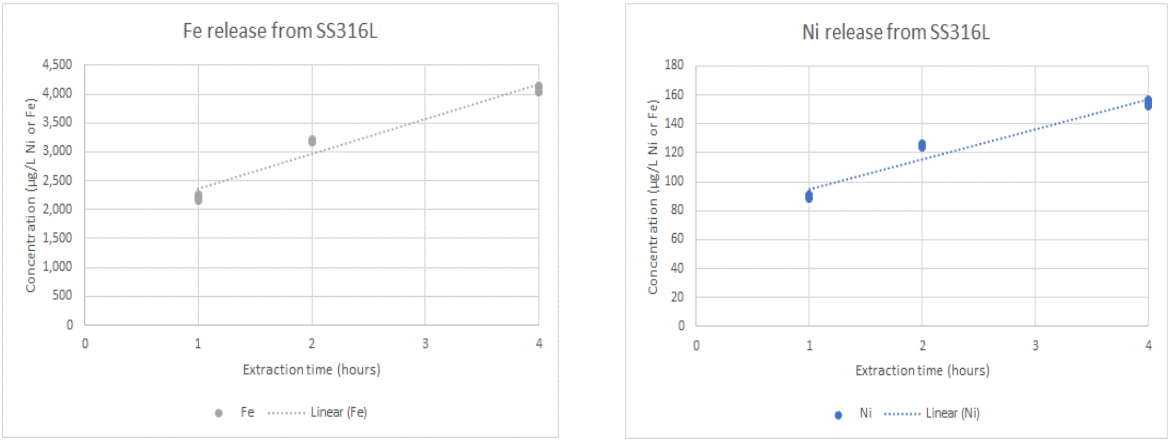


Figure 3: Time-dependent metal release in gastric fluid for SS316L (powder form) (ECTX 2018b).

As could be expected, Figure 2 indicates that the two water soluble metal compounds were fully dissolved within the first hour in gastric fluid. By contrast, the release of Fe and Ni from the metal samples was reasonably linear with time between 1 and 4 hours. No saturation of metal release is observed at the 2 h extraction time. Likewise, Figure 3 indicates that no saturation is observed for metal releases from the alloy SS316L.

The following massive samples (as received, representative of materials on the market) were tested: an alloy (Z45, 62.176% copper) and its pure ingredient (Cu^0, 100% Cu), both as disks. Loadings of 2 g/L surface equivalent were tested (ECTX 2018c). Triplicate sample vessels with duplicate sampling from each vessel were included. Figure 4 shows the copper releases as a function of time.

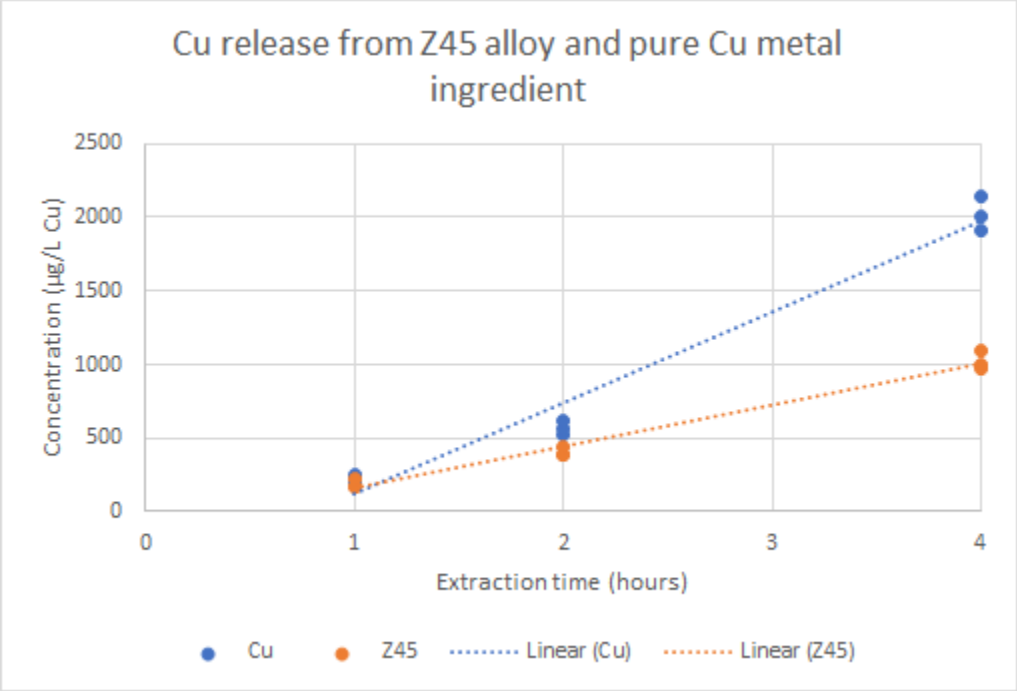


Figure 4: Time-dependent Cu release in gastric fluid from Z45 and pure copper metal (massive forms)(ECTX 2018c).

The results indicate that for massive forms of alloys, as for powders, no saturation of metal release after 2 hours in gastric fluid is evident for the alloy examples tested.

Further details about these studies, as well as the raw bioaccessibility data and the calculated (mg/g sample) values can be found in the following ECTX reports:

- ECTX. 2016: **X02a-084**: 2 hours bioelution study on stainless steel 316L powder at a 2 g/L loading in simulated gastric fluid
- ECTX. 2017a. **X02a-130**: A 2 hours bioelution Study on Fe-Cr-Ni Powder Mixture (68.6/16.4/10.7 mass fraction) at a *0.2, 1 and 2 g/L loading* in a simulated gastric fluid.
- ECTX. 2018a. **X02a-144**: 2 hours bioelution study on stainless steel 316L powder at a 2 g/L loading in simulated gastric fluid- effect of mass sample.
- ECTX. 2018b. **X02a-145**: 4 hours bioelution kinetics study on stainless steel 316L powder at a 2 g/L loading in simulated gastric fluid.
- ECTX. 2018c. **X02a-146**: 4 hours bioelution kinetics study on Z45 (massive) at a 2 g/L loading (surface equivalent) in a simulated gastric fluid

These reports are available from Eurometaux (and NiPERA for ECTX 2016) upon request.