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Bioaccessibility of metals in alloys: Evaluation of three surrogate biofluids

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ABSTRACT

Bioaccessibility *in vitro* tests measure the solubility of materials in surrogate biofluids. However, the lack of uniform methods and the effects of variable test parameters on material solubility limit interpretation. One aim of this study was to measure and compare bioaccessibility of selected economically important alloys and metals in surrogate physiologically based biofluids representing oral, inhalation and dermal exposures. A second aim was to experimentally test different biofluid formulations and residence times *in vitro*. A third aim was evaluation of dissolution behavior of alloys with *in vitro* lung and dermal biofluid surrogates. This study evaluated the bioaccessibility of sixteen elements in six alloys and 3 elemental/metal powders. We found that the alloys/metals, the chemical properties of the surrogate fluid, and residence time all had major impacts on metal solubility. The large variability of bioaccessibility indicates the relevancy of assessing alloys as toxicologically distinct relative to individual metals.

1. Introduction

Alloy dust is a byproduct of the manufacturing and use of alloy materials such as stainless steel (SS). Occupational workers and to a lesser extent the general public are exposed to alloy and metal mixtures in dust. Normally regulatory agencies attempt to define and regulate risk of mixtures relative to the sum of the individual components (U.S.EPA, 2000; Vyskocil et al., 2004) but metal alloys are a unique class of substances defined as "consisting of two or more elements so combined that they cannot be readily separated by mechanical means" (Skeaff et al., 2007; UNGHS, 2005). There are many alloys and each exhibits unique properties. Studies highlighting intrinsic differences in the solubility of metals in various alloys (Flint, 1998; Herting et al., 2008b; Skeaff et al., 2007; Stopford et al., 2003) recognize the significance of their unique qualities. These studies indicate the importance of testing metal alloys separately from metal ores or minerals when classifying hazard.

Biologically relevant exposure tests measuring the chemical dose that is available for uptake are gaining greater attention and support for public health applications (Birnbaum, 2010). One such exposure test, the bioaccessibility *in vitro* test, has been used to account for the relative bioavailability of contaminants in human health risk assessments (Brandon et al., 2006; Brock and Stopford, 2003; EN, 2009; Henderson et al., 2012; U.S.EPA, 2007). Bioaccessibility is an important facet of bioavailability, and it is frequently defined as the biologically relevant fraction of a chemical that is potentially available for uptake into a biological organism (Anderson and Hillwalker, 2008; Brandon et al., 2006; Ruby et al., 1999). The test only provides an estimate of the complex physiological and physicochemical processes that occur in human toxicokinetics, but represents the step in bioavailability that is most sensitive to the chemical behavior of materials (Brandon et al., 2006; Drexler and Brattin, 2007).

Bioaccessibility *in vitro* tests, bio-elution, offer the advantages of simplicity, speed, affordability and ethical considerations over *in vivo* bioassays. Human surrogate biofluids used in bioaccessibility tests include gastro-intestinal (saliva, stomach, intestine), dermal (sweat), lung (alveolar, interstitial, lysosomal, serum) and internal implantation (lysosomal/cytosol). Oral bioaccessibility

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tests are the most frequently investigated with the test methodology ranging from a static gastric compartment (Drexler and Brattin, 2007; EN, 2002; Stopford et al., 2003; U.S.EPA, 2007) to dynamic gastrointestinal models (Garcia et al., 2001; Juhasz et al., 2009; Rodriguez and Basta, 1999; Ruby et al., 1996; Velasco-Reynold et al., 2008). While multiple gastric methods persist, alloy dermal biofluid studies have generally used the EN 1811 reference method for allergenic responses via skin contact (Bocca et al., 2007; Flint, 1998; Julander et al., 2009; Midander et al., 2007b). However, far fewer studies have applied *in vitro* bioaccessibility tests to lung (Herting et al., 2008b; Midander et al., 2007b; Stopford et al., 2003; Thelohan and Demeringo, 1994; Twining et al., 2005; Vitarella et al., 2000) or internal implantation (Herting et al., 2008a; Stopford et al., 2003) biofluids to assess inhalation exposure to alloys. A critical barrier to this type of testing is the lack of standardization for selecting physiologically-based extraction conditions including residence time, substance mass to biofluid volume ratio, agitation, and biofluid formulation chemistries. While some test parameters have been more thoroughly investigated, such as particle size, (Hedberg et al., 2010; Midander et al., 2007a) test mass to biofluid ratio (Hamel et al., 1998; Thelohan and Demeringo, 1994), and agitation (Midander et al., 2006), other method variations, such as biofluid formulation and effect of residence time, are not as well characterized for use with alloys. These parameters are often manipulated between studies, making it difficult to compare bioaccessibility results and further preventing incorporation of a bioaccessibility test into health risk characterization.

This study evaluates metal bioaccessibility from several economically important grades of alloys in physiologically based *in vitro* biofluids representing three major exposure routes: gastric, lung and dermal. Biofluid formulations and residence times are two commonly employed test parameters that were evaluated using standard alloy reference materials. We illustrate that the *in vitro* bioaccessibility tests are applicable to assessing unique qualities of different alloy grades for health characterization purposes. We measure dissolution rates for nine alloys/metal powders in two biological surrogate biofluids. Six alloys and three elemental metal powders are compared using the major exposure route surrogate biofluids: gastric, lung and dermal.

2. Materials and methods

2.1. Materials

Four commercially available austenitic steel alloys and three metal powders were purchased from Atlantic Equipment Engineers (NJ, USA). The alloys included the American Iron and Steel Institute (AISI) stainless steel (SS) grades 316 and 304; the Ni–Cr Inconel and Ni–Cu Monel superalloys; and the metal powders included cobalt, manganese and nickel. Two alloy standard reference materials (SRMs) were purchased though National Institute of Standards and Testing (NIST, Gaithersburg, MD); SRM 101g (stainless steel, SS 304L) and SRM 14g (carbon steel). Table 1 lists the physical and chemical compositions of the alloys and metal powders.

2.2. Surrogate biofluids

Three human surrogate biofluids representing those involved in oral (gastric), inhalation (artificial lysosomal fluid [ALF]) and dermal (sweat) human exposure pathways were selected to measure alloy bioaccessibility. Different chemical formulations of the individual biofluids have been reported (Hedberg et al., 2010; Herting et al., 2007; Stopford et al., 2003), however, the effects of different biofluid formulations have not received adequate attention. To evaluate the magnitude of the effects, two commonly reported versions of ALF and two versions of gastric biofluids were applied to the SRM alloys.

Gastric biofluids from the static gastric compartment model are simple surrogates with low pH levels (pH 1.2–1.5) representing a worst-case fasting exposure scenario for a conservative bioaccessibility assessment (Brock and Stopford, 2003; EN, 2009; Juhasz et al., 2009; U.S.EPA, 2007). Two different gastric solution compositions were selected; a 0.07 N HCl solution further developed by Stopford et al. for determining metals in art material (ASTM, 2007; Stopford et al., 2003) and an approximately 1 N HCl solution buffered with 2.5 M glycine (herein described as gastric–GLY) used by the US EPA to assess gastric bioaccessibility of lead in soil (Drexler and Brattin, 2007). This oral bioaccessibility model was selected because the static approach has undergone extensive inter-laboratory round robin testing (ASTM, 2007; Drexler and Brattin, 2007; EN, 2002; U.S.EPA, 2009) and validation with *in vivo* studies with soil matrices (Rodriguez and Basta, 1999; U.S.EPA, 2007).

ALFs are composed of complex salts and organic acids with low pH (pH 4.5) simulating phagocytosis of particulates by lung alveolar cells and interstitial macrophages (Stopford et al., 2003; Thelohan and Demeringo, 1994) and inflammatory response connected with surgical implants in the body (Herting et al., 2008a). Two ALF compositional differences reported in the literature include either the use of glycine (Thelohan and Demeringo, 1994) or an equivalent mass of glycerol (Stopford et al., 2003), herein described as glycine–ALF and glycerol–ALF, respectively. Surrogate sweat (pH 6.4–6.6) that used was prepared according to the EN 1811 standardized test, which is commonly used for allergenic response from nickel, chromium, cobalt and other metals in alloys (Bocca et al., 2007; EN, 2009; Julander et al., 2009; Midander et al., 2007b).

The complete compositions of the five biofluids used are listed in Supplementary information (SI) Table S1. All solutions were prepared using 18 MΩ cm water and analytical grade reagents and chemicals. ALF and sweat were used within a week and 3 h of preparation, respectively. The gastric fluids were considered stable throughout the study duration.

2.3. Experimental conditions

Test parameters evaluated included multiple formulations of biofluids and three residence times. Two formulations each for gastric and lung biofluids were tested. Gastric was tested with and without glycine ($C_2H_5NO_2$) and ALF was tested with glycine or glycerol ($C_3H_8O_3$). Complete compositions of all test biofluids are described in SI Table SI. Three residence times, 2, 24 and 72 h, were tested for lung and dermal biofluids and two residence times, 2 and 72 h were tested for gastric solutions, Table 2 and Fig. 1. The gastric and glycerol–ALF formulations were used for the residence time studies, Table 2 and Table S1. All test parameters were performed with two SRMs, carbon steel (NIST 14g) and stainless steel (NIST 101g), which represent vastly different alloys.

We then evaluated the bioaccessibility of 4 alloys and 3 elemental metal powders in the following biofluids: gastric, lung and dermal (Table 3). Here we focused on one formulation and one residence time for each biofluid. Gastric employed HCl for 2 h, lung utilized ALF with glycerin for 72 h, and dermal sweat was tested as described above for 72 h.

The preparation consisted of 0.1 g (±10%) of test alloy/metal powder with 50 mL of surrogate biofluid representing a 1:500 g/mL extraction ratio. This exposure ratio

Table 1
Chemical composition (wt%) and particle size of test materials.

Test material (grade[a])	Co	Cr	Cu	Fe[b]	Mn	Ni	Mo	P	Particle size[c]
Carbon steel 1078 (NIST 14g)	0.0030	0.0810	0.0470	–	0.4560	0.0300	0.0110	0.0060	0.5–1.18 mm
Stainless steel 304L (NIST 101g)	0.0900	18.46	0.0290	–	0.0850	10.0	0.0040	0.0070	75–710 μm
Stainless steel 304	0.09	18.02	0.0290	68.30	0.15	11.14	–	0.012	44–149 μm
Stainless steel 316	–	16.74	–	69.58	0.08	11.69	2.15	0.03	44–149 μm
Inconel (Ni–Cr)	–	15.78	0.500	9.000	0.07	74.19	–	–	<44 μm
Monel (Ni–Cu)	–	–	28.9	0.080	2	67.11	–	–	<44 μm
Co metal	99.8	–	–	–	–	–	–	–	<36 μm
Mn metal	–	–	–	–	99.8	–	–	–	44–297 μm
Ni metal	–	–	–	–	–	99.8	–	–	44–149 μm

[a] American Iron and Steel Institute.
[b] Approximate iron balance.
[c] Sieve analysis.