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Determination of the bioaccessible fraction of metals in urban aerosol using simulated lung fluids

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HIGHLIGHTS

- Simulated lung fluids used in determination of metals bioaccessibility were compared.
- A new fluid based on DPPC, "Simulated Alveoli Fluid" (SAF), was proposed.
- Fluids with decreased surface tension provided the lowest extraction yields.
- Fluids with low surface tension are recommended despite lower extraction efficiency.

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ABSTRACT

Determination of the bioaccessible fraction of metals in atmospheric aerosol is a significant issue with respect to air pollution in the urban environment. The aim of this work was to compare of metal bioaccessibility determined according to the extraction yields of six simulated lung fluids. Aerosol samples of the PM1 fraction were collected in Brno, Czech Republic. The total contents of Cd, Ce, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn in the samples were determined and their enrichment factors were calculated. The bioaccessible proportions of elements were determined by means of extraction in Gamble's solution, Gamble's solution with dipalmitoyl phosphatidyl choline (DPPC), artificial lysosomal fluid, saline, water, and in a newly proposed solution based on DPPC, referred to as "Simulated Alveoli Fluid" (SAF). The chemical composition and surface tension of the simulated lung fluids were the main parameters influencing extraction yields. Gamble's solutions and the newly designed solution of SAF exhibited the lowest extraction efficiency, and also had the lowest surface tensions. The bioaccessibility of particulate metals should be assessed by synthetic lung fluids with a low surface tension, which simulate better the behavior and composition of native lung surfactant. The bioaccessibility of metals in aerosol assessed by means of the extraction in water or artificial lysosomal fluid can be overestimated.

1. Introduction

Pollution of the urban environment caused by transport, industry, and fuel combustion entails considerable health risks associated with increased contents of particulate matter (PM) in the atmosphere. Elevated concentrations of PM relate to an increase in respiratory and cardiovascular mortality and proinflammatory effects. Pulmonary inflammation and altered respiratory immune responses are indications of reactive oxygen species induced by metals (Sun et al., 2001; Wiseman and Zereini, 2014).

Submicron particles (i.e. particles with an aerodynamic diameter smaller than 1 μm, PM1) enter into the alveoli (Mukhtar and Limbeck, 2013); ultrafine particles (i.e. particles with an aerodynamic diameter smaller than 0.1 μm) can be adsorbed onto the pulmonary epithelium (Brunekreef and Holgate, 2002). The epithelium of the alveoli is covered with lung surfactant, which plays a key role in correct lung function (Barrett et al., 2010; Goerke, 1998; Pérez-Gil and Keough, 1998; Rooney et al., 1994). The interaction of PM with lung surfactant in alveoli can be expected, including dissolution, phagocytosis, and membrane transfer into the bloodstream (Brunekreef and Holgate, 2002; Sun et al., 2001). Lung surfactant consists primarily of phospholipids and proteins; the main component of the surfactant is phosphatidyl choline, namely dipalmitoyl phosphatidyl choline (DPPC) (Barrett et al., 2010; Blanco and Pérez-Gil, 2007). DPPC is the main active component lowering the surface tension of lung fluid. Moreover,

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active alveolar films appear to be enriched with DPPC, when considering the DPPC concentration in bronchoalveolar lavage (Goerke, 1998). The efficiency of native or synthetic surfactants used in the treatment of Respiratory Distress Syndrome, which may occur in premature infants, also depends on the presence of other substances, such as neutral lipids and proteins (Blanco and Pérez-Gil, 2007; Pérez-Gil and Keough, 1998). The contents and functions of organic substances in the lung surfactant are known (Blanco and Pérez-Gil, 2007). In contrast, the influence of inorganic salts has been little investigated.

The health risks posed by inhaled particles are associated not only with the chemical composition and solubility of particle components but also with their speciation composition and with the overall level of exposure to PM. The most toxic metallic components of PM are soluble species, which can be more readily bioactivated (Mukhtar and Limbeck, 2013). The total metal content in PM is not sufficient for health risks to the population to be estimated accurately; determination of the bioaccessible proportion is preferable. Extraction in simulated lung fluids (SLFs) is used to assess the bioaccessibility of metals in PM (Mukhtar and Limbeck, 2013). Moss (1979) reported that the composition of artificial lung surfactant used in extraction should be as close as possible to that of real surfactant. However, traditionally used solutions, such as Gamble's solution or artificial lysosomal fluid (ALF), are composed of various salts that are intended to substitute the complex composition of lung surfactant (Stopford et al., 2003). Thus, the extraction of PM characterizes, in particular, the solubility of elements in the extractant comprising water, weak acids, chelating agents, buffers, and inorganic salts (Mukhtar and Limbeck, 2013) at physiological pH and temperature (Barrett et al., 2010).

This work aims to estimate the bioaccessibility of metals in urban aerosol (PM1) by a critical comparison of the extraction efficiencies of SLFs in relation to their surface tension. Of particular interest was the proposal of a new SLF suitable for the bioaccessibility determination of metals.

2. Materials and methods

2.1. Collection of PM1 samples

Samples of urban aerosol (size fraction PM1) were collected in Brno (49° 12′ N, 16° 36' E), Czech Republic. The city is burdened by heavy traffic, with a high number of days each year in which PM exceeds its legal limits (Coufalík et al., 2014). Sampling was carried out on the terrace of the Institute of Analytical Chemistry at a height of 8.9 m, 15.6 m away from the road surface. Due to the open profile of the street, an accumulation of metals at the sampling point could not be expected, as opposed to the street canyon (Coufalík et al., 2014).

In total, 16 samples of urban PM1 aerosol were collected. Samples No. 1–8 were collected in August/September 2014 (4 samples during the working week and 4 samples during the weekend), samples No. 9–16 were collected in January/February 2015 (also 4 samples during the working week and 4 samples during the weekend). The collection was performed by a high-volume sampler (DHA-80, Digitel, Switzerland) with a PM1 size selective inlet. Cellulose nitrate membrane filters (150 mm diameter, 3 μm porosity, Sartorius) were equilibrated before sampling in a clean climatic room at R.H. 50% (±2%) and 20 °C (±1 °C) for 48 h. Subsequently, they were weighed on an M5P microbalance (±1 μg, Sartorius, Germany) and sealed in Petri dishes with Parafilm until sampling. The sampling of PM1 aerosol was carried out at an air flow rate of 30 m^3/h for 48 h. After the sampling, filters were equilibrated, weighed, and stored in Petri dishes at 4 °C in the fridge until analysis. Four unsampled filters were analyzed together with the samples as process blanks.

2.2. Simulated lung fluid preparation and filter extraction

Deionized water with a conductivity of 0.055 μS/cm was used for the preparation of all extraction solutions (Ultra Clear UV Plus TM, Evoqua Water technologies, Germany). Sub-boiling in-house distilled nitric acid was used for the total decomposition of samples and the stabilization of extracts. Solutions of SLFs were prepared according to the recipes in Table 1. All chemicals were for trace analysis (if available). When preparing the solutions, it was necessary to add the constituent chemicals in the correct order and into a sufficient volume of water to avoid any precipitation.

The following SLFs were used for the extractions of filters: (A) Gamble's solution, which has been suggested as a model lung interstitial fluid for *in vitro* testing (Moss, 1979), and (B) Artificial alveolar fluid, which is Gamble's solution with 1,2-Dipalmitoyl-sn-Glycero-3-Phosphatidylcholine (DPPC) (Stopford et al., 2003) – the pH of these two solutions is consistent with the physiological pH in lungs (pH = 7.4) (Barrett et al., 2010); (C) Artificial lysosomal fluid (C), which simulates the phagocytosis of alveolar and interstitial macrophages and has a pH of 4.5–5.0 (Stopford et al., 2003); and (D), a new solution proposed in this study, "Simulated Alveoli Fluid" (SAF), which attempts to simulate extraction in alveoli. SAF is composed of DPPC, Ca^{2+} ions, and buffer at a pH of 7.4. Extractions of PM1 samples in saline (E) and deionized water (F) were also performed.

Before analysis, the filters were equilibrated in a climatic room for 48 h and then weighed. Subsequently, each filter was divided into 7 parts with a ceramic knife on a PTFE plate and each part was weighed. One part was used to determine total metal contents in a sample (after decomposition in HNO_3); the remaining six parts were extracted in six different SLFs.

The decomposition of samples was performed by an UniClever microwave mineraliser (Plazmatronika, Poland). One seventh of each filter was decomposed in 6 mL of sub-boiling HNO_3 for 15 min. The decomposed samples were transferred quantitatively along with 6 mL of deionized water into polyethylene scintillation vials (Kartel, Italy).

The extractions of the remaining six filter parts were performed by means of a reciprocating shaker in a thermostated box at 37 °C (±0.5 °C) for 24 h. Each filter part was extracted in 20 mL of extractant in glass vials (a volume of 50 mL). After extraction, the extracts were filtered through a polyethersulfone syringe filter (0.45 μm) into the polyethylene scintillation vials (Kartel, Italy). The extracts were stabilized by the addition of 100 μL of sub-boiling HNO_3 per 10 mL of solution. The samples were stored at 4 °C.

All operations were carried out in a clean laboratory with HEPA filters. Substantial attention was paid to the avoidance of extraneous contamination, including the cleaning of glass in HNO_3 for at least 24 h and subsequent leaching in deionized water, and the boiling of Teflon cups in HNO_3.

2.3. Analytical procedures

Metal contents in SLF extracts and samples decomposed in HNO_3 were analyzed using an Agilent 7700x ICP-MS spectrometer with ASX-500 autosampler. A robust tune (1600 W power, 0.6 L/min of Ar carrier gas, and 0.55 L/min of Ar dilution gas) was used throughout. ^{27}Al, ^{51}V, ^{52}Cr, ^{55}Mn, ^{57}Fe, ^{60}Ni, ^{63}Cu, ^{65}Cu, and ^{66}Zn were measured in collision mode with 4.8 mL/min of He, while ^{111}Cd, ^{140}Ce, ^{206}Pb, ^{207}Pb and ^{208}Pb were measured in standard (no gas) mode. A solution of 20 ng/mL of ^{45}Sc, ^{89}Y and ^{159}Tb was used as the internal standard.