Chemosphere 158 (2016) 124–130

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Evaluating the mutagenicity of the water-soluble fraction of air particulate matter: A comparison of two extraction strategies

Isabel C. Palacio [a, b], Ivo F. Oliveira [a], Robson L. Franklin [a], Silvia B.M. Barros [b], Deborah A. Roubicek [a, *]

[a] Dept. Environmental Analyses, São Paulo State Environmental Agency, CETESB, Av. Prof. Frederico Hermann Jr, 345, 05459-900, São Paulo, SP, Brazil
[b] Dept. Clinical Analyses and Toxicology, Faculty of Pharmaceutical Sciences, University of São Paulo, Av. Professor Lineu Prestes, 580, 05508-000, São Paulo, SP, Brazil

HIGHLIGHTS

- We compared microwave-assisted and ultrasonic bath extractions of airborne PM.
- Extraction method can significantly affect the results of mutagenicity of PM.
- Metals associated with airborne PM have mutagenic potential (Ames test).
- Based on the biological response, MW is suitable and efficient extraction method.
- Water-soluble extracts can be frozen up to 60 days before testing.

ARTICLE INFO

Article history:
Received 29 March 2016
Accepted 19 May 2016
Available online 31 May 2016

Handling Editor: Jianying Hu

Keywords:
Extraction
Genotoxicity
Air particulate matter
Water-soluble fraction

ABSTRACT

Many studies have focused on assessing the genotoxic potential of the organic fraction of airborne particulate matter. However, the determination of water-soluble compounds, and the evaluation of the toxic effects of these elements can also provide valuable information for the development of novel strategies to control atmospheric air pollution.

To determine an appropriate extraction method for assessing the mutagenicity of the water-soluble fraction of PM, we performed microwave assisted (MW) and ultrasonic bath (US) extractions, using water as solvent, in eight different air samples (TSP and PM10). Mutagenicity and extraction performances were evaluated using the *Salmonella*/microsome assay with strains TA98 and TA100, followed by chemical determination of water-soluble metals. Additionally, we evaluated the chemical and biological stability of the extracts testing their mutagenic potential and chemically determining elements present in the samples along several periods after extraction. Reference material SRM 1648a was used.

The comparison of MW and US extractions did not show differences on the metals concentrations, however positive mutagenic responses were detected with TA98 strain in all samples extracted using the MW method, but not with the US bath extraction. The recovery, using reference material was better in samples extracted with MW. We concluded that the MW extraction is more efficient to assess the mutagenic activity of the soluble fraction of airborne PM. We also observed that the extract freezing and storage over 60 days has a significant effect on the mutagenic and analytical results on PM samples, and should be avoided.

1. Introduction

Many epidemiological studies have demonstrated the association between exposure to airborne particulate matter (PM) and adverse human health effects as lung cancer and cardiovascular diseases at concentrations commonly found in urban areas around the world (Boffetta, 2006; Kim et al., 2015; Silva et al., 2010).

PM consists of a complex mixture of different organic and

* Corresponding author.
E-mail addresses: icpb@usp.br (I.C. Palacio), ifoliveira@sp.gov.br (I.F. Oliveira), rfranklin@sp.gov.br (R.L. Franklin), smbarros@usp.br (S.B.M. Barros), droubicek@sp.gov.br (D.A. Roubicek).

http://dx.doi.org/10.1016/j.chemosphere.2016.05.058
0045-6535/

inorganic compounds, many of which have mutagenic, genotoxic and/or carcinogenic capacity (Baja et al., 2010; Ceretti et al., 2015; Cheng et al., 2004; Pope et al., 2002; Roubicek et al., 2007). The mutagenicity of the airborne particulate matter has been linked to approximately 500 compounds of different chemical classes. Among these, the best known are the polycyclic aromatic hydrocarbons (PAH). In contrast, few studies have focused on investigating the water soluble fraction of the particulate matter and the differential contribution of different chemical species to the genotoxic and mutagenic damage (Claxton et al., 2004).

Because of the complex chemical nature of PM, standard chemical analyses are limited in their ability to characterize the chemical composition of these mixtures and allow the identification of potential genotoxic substances present in the samples (Claxton et al., 1998). The combination of short-term bioassays with chemical-analytical techniques have been successfully used to identify a variety of mutagenic compounds in complex matrices such as air. The bioassays are valuable tools because they allow the detection of the effects of several chemicals and their interactions, even when they occur at low concentrations (Kessler et al., 2012). The *Salmonella*/microsome assay is one of the most valuable tools for identifying the presence of mutagens in the environment and it is considered an essential bioassay for environmental monitoring studies aimed at genotoxic or cancer risk assessment (Villalobos-Pietrini et al., 2006).

Toxicological studies implicated the metal content (particularly water-soluble metal) as a possible harmful component of PM (Shuster-Meiseles et al., 2016; Wiseman, 2015). Metals associated with PM may be in highly mobile forms (water-soluble) and, therefore, potentially bioavailable (Fernández Espinosa et al., 2002; Mbengue et al., 2015). Toxic metals can be absorbed in lung tissue during breathing influencing biological functions (Lemos et al., 2012).

Although genotoxic positive results caused by metals linked to the inorganic soluble fraction of the PM have been reported in literature (Dellinger et al., 2001; Gutiérrez-Castillo et al., 2006; Mukherjee et al., 2004; Valavanidis et al., 2000), the determination of the mutagenic effects using the *Salmonella*/microsome assay with water as sole solvent is still uncommon. Traditionally, a combination of acids and oxidizing agents (HNO_3, HF, H_2O_2, and H_3BO_3) is used in the extraction process of the inorganic fraction of the particulate matter, in order to chemically quantify the total metal content, and in the determination of oxidative damage induced by soluble metals on the DNA. This methodology cannot be applied to the *Salmonella*/microsome assay and other biological systems, like cell cultures, due to its high sensitivity to the acidification of the sample.

Because the toxicity of the particulate matter is influenced by the solubility of metals and its reactive capacity, determining the content of soluble metals and their mutagenic potential in particulate matter samples by simple and reliable methods is important to understand their impact on human health and the environment. The method of extraction can significantly affect the results of mutagenicity testing of complex mixtures, as the preparation of these complex samples is a critical step in the analytical procedure. Approximately 55–95% of the inter-laboratory variability in the results of mutagenicity tests of particulate matter samples is due to differences in analytical methods rather than real differences in the mutagenic potential of the samples (Marvin and Hewitt, 2007). In recent years there has been an increased search for new, quick, and more efficient extraction techniques as a result of the large number of PM samples collected and analyzed in the networks of monitoring the air quality (Sathrugnan and Balasubramanian, 2005). Usually, ultrasonic baths are used to extract substances from particulate matter; however microwave radiation assisted procedures are increasingly more used for preparing the samples (Karthikeyan et al., 2006).

This study aimed to compare the efficiency of microwave-assisted and ultrasound bath in the extraction of the soluble fraction of particulate matter, using water as solvent and the *Salmonella*/microsome assay to measure the mutagenic response. The efficiency of the extraction method was evaluated using standard reference material SRM 1648a based on recovery results, and the chemical and biological stability of the water-soluble fraction of the particulate material was also evaluated.

2. Material and methods

2.1. Air particulate matter samples

A total of four samples of total suspended particles (TSP) and four samples of coarse inhalable particles (PM10) were analyzed in order to compare the extraction methods. TSP and PM10 samples were collected in glass fiber filters (Pall Corporation type A/E, 20.3 cm × 25.4 cm) with high volume samplers (General Metal Works INC/Energetica, respectively) operating with a sampling flow of 1.13 m^3/min for 24 h, every six days, by the São Paulo State Environmental Agency (CETESB), as part of the regular air quality monitoring program. The collected material was determined gravimetrically. The filters were dried for 48 h in a desiccator under a temperature of 15–30 °C, controlled relative humidity and were weighed before and after collection. The TSP and PM10 concentration was expressed in μg/m^3 of air sampled. The filters were stored at room temperature and protected from light until analysis. All samples were collected in different places in the state of São Paulo that have an industrial economic vocation. For the purpose of this study, samples were pooled together regardless the site they were collected.

2.2. Extraction procedures

The samples were submitted to extraction using ultrapure water as sole solvent. Each sample consisted of a pool of ¼ of 4 filters, and the total mass from each sample was calculated by the sum of ¼ of the mass of all filters belonging to that sample. Although the mass of each filter was different, we extracted all pools with the same amount of water relative to PM mass in order to get 2 ml of the extract as a maximum dose in the *Salmonella* assay.

2.2.1. Microwave-assisted (MW) extraction

The extraction of the water-soluble substances contained in the particulate matter was performed in a Milestone microwave, model Ethos 1 (Sorisole-BG, Italy), medium pressure system, for up to 12 tubes of TFM®. The filters were placed in the tubes with 20 ml of ultrapure water (18.2MΩ) (Purelab Ultra SC MK2 Elga, WS- England). The equipment was set to a power of 1000 W and a temperature of 100 °C for 10 min with a heating ramp-up for 10 min. After cooling, the suspension was filtered in a 0.45 μm Millipore membrane filter (Billerica, MA-USA).

2.2.2. Ultrasonic bath (US) extraction

The filters were fractionated and deposited in an Erlenmeyer flask with 40 ml ultrapure water. The samples were agitated in a shaker (SOLAB SL, 223, SP-Brazil) for 1 h at 160 rpm at 36 °C and placed in an ultrasonic bath for 30 min at room temperature. This procedure was repeated twice for each sample. We used a Unique USC-1800 Ultrasonic bath (São Paulo, SP, Brazil) (40 KHz, 154 W). After extraction, the sample was passed through a 0.45 μm Millipore membrane filter (Billerica, MA-USA).

Additionally, clean control filters were treated in the same way