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A high-efficiency, low-bias method for extracting particulate matter from filter and impactor substrates

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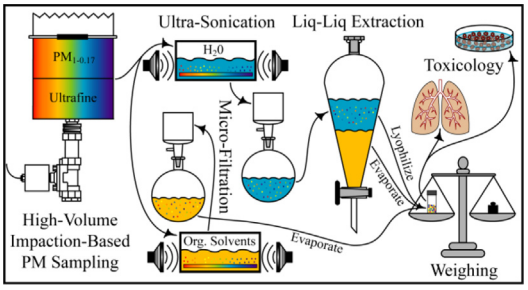
HIGHLIGHTS

- Novel PM extraction methods are presented that:
- Maximize extraction efficiency and minimize artifacts and compositional biases.
- Achieve 10–40% efficiency increase for ultrafine PM extracted from afterfilters.
- Achieve 20–50% efficiency increase for submicron fine PM extracted from PUF.
- Demonstrate that extraction efficiencies are compositionally, or source, specific.

GRAPHICAL ABSTRACT



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ABSTRACT

Atmospheric particles are frequently collected onto filter and impactor substrates for studies related to the composition, health effects and climate impact of ambient particulate matter (PM). Many of these studies require extraction of that PM from the substrates but available methods have low extraction efficiencies that may lead to compositional and thus toxicity bias. Here, novel PM extraction methods are presented that *(a)* maximize extraction efficiency, *(b)* minimize compositional biases in extracted PM, relative to sampled PM and *(c)* minimize extraction artifacts. Method development was based upon strengths and weaknesses of existing SOPs and current requirements in the field of aerosol health effects research. Extraction objectives were accomplished using a combination of sonication in solvents of varying polarity, selective filtration, liquid–liquid extraction of water-based extracts, solvent removal and final reconstitution of the total extracted PM. Relying largely on intensive gravimetric analyses and comparison to existing SOPs, the new technique has been fully validated on nearly 40 different size-segregated, source-oriented samples collected during two separate seasons in Fresno, CA. Compared to existing methods, and depending on the source, compositionally-specific increases in extraction efficiencies of 10–40% and 20–50% were obtained for the ultrafine and submicron fine PM fractions, respectively, indicating significant increases in total extraction efficiency and significant decreases in compositional bias.

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1. Introduction

Epidemiological studies associate gas and particle pollutant concentrations with a range of human health effects, but these associations rely on follow-up toxicological studies to validate the epidemiological associations and establish their cause, effect and underlying mechanism. As a result, numerous stakeholder agencies such as the U.S. Environmental Protection Agency (EPA), National Institute of Environmental Health Sciences (NIEHS) and California Air Resources Board (CARB) employ toxicology studies in animal models and in vitro systems to assess the toxicity and relative toxicity of atmospheric particulate matter (PM). The PM for such studies is typically collected from the atmosphere via high-volume, impaction-based filter sampling and then subsequently extracted from these filters and impaction substrates into water or other media for toxicological assessment. The primary goal of filter extraction methods for toxicological studies is to conserve the physical and chemical properties of the PM as it originally existed in the atmosphere – including particle size, number concentration, morphology and individual particle compositional and structural integrity – so that the results of these studies are representative of true population exposure.

Unfortunately, the physical integrity of PM, e.g. particle size distribution and morphology, is mostly lost during impaction-based sampling as a result of aggregation; i.e. particles stick together due to high speed collisions as particles impact and collect on the filter and impactor substrates. Single particle compositional and structural integrity is further obscured by the use of solvents (i.e. water) during filter extraction that dissolve certain PM components, such as inorganic salts, that are then redistributed during solvent removal. Efforts can be made to recapture these properties as much as possible but the original state of the PM will never be fully recreated. As a result, the best effort imperatives of any filter extraction method become conserving total PM mass and bulk chemical composition, which is achieved by *(a)* maximizing extraction efficiency, i.e. the ratio of extracted PM mass to the total PM mass collected on the filter, *(b)* maximizing relative extraction effectiveness as a function of particle size fraction and chemical category – e.g. minerals, metal oxides, salts/electrolytes, acids/bases, hydrophobic/hydrophilic organic compounds, polycyclic aromatic hydrocarbons (PAHs) and black/brown carbon – and *(c)* minimizing extraction artifacts such as volatilization losses, chemical alterations to the PM and incorporation of contaminant filter material or solvents into the extracted PM sample.

Standard operating procedures (SOPs) for performing filter extractions are available from various groups, including the EPA. Different groups employ different SOPs, potentially resulting in outcomes that are partially or wholly dependent on the SOP employed and thus biasing inter-group comparisons. Remarkably, there has never been a systematic study published in the literature that investigates this potential bias or compares the various SOPs in terms of the imperatives listed above. In the current work, we have designed, developed and deployed novel extraction techniques that build on the strengths of existing SOPs by finding approaches that mitigate their weaknesses and increase efficiency while also addressing the concerns of the toxicology community. The main impetus of these efforts has been the general paucity of existing literature and a specific need to develop methods that maximize extraction efficiencies while minimizing potential compositional biases and extraction artifacts.

In previous work, we designed and successfully deployed a novel sampling technique that allowed us to collect size-segregated, source-oriented PM from the ambient mixture (Bein et al., 2009). Briefly, the system conditionally samples particles from the atmosphere by identifying particle sources in real-time using a single particle mass spectrometer (RSMS) (Bein et al., 2005). The RSMS then controls a bank of 10 ChemVol (CV) samplers (Demokritou et al., 2002) where each CV is assigned a specific source or source combination and RSMS controls which CV operates depending on the sources it currently observes. CVs are high flow rate (900 lpm), impactor-based samplers with multiple impactor stages that can be stacked in series to collect size-segregated PM for a range of size cuts. In the current design, each CV stack includes an afterfilter support and 0.17 and 1 μm stages so that ultrafine (UF; $D_p < 0.17$ μm) and submicron fine (SMF; $0.17 < D_p < 1$ μm) fractions are collected for each source. This system was operated for several months during two separate experiments in Fresno, CA, resulting in the collection of 18 size-segregated, source-oriented samples during summer 2008 (S08) and 20 during winter 2009 (W09). The afterfilters were constructed from PTFE bonded borosilicate glass microfibers reinforced with woven glass cloth and used to collect UF PM. The SMF impaction substrates were composed of polyurethane foam (PUF). PM was extracted from both substrates using the techniques described in the following section. The results from these extractions are then compared and contrasted to existing SOPs.

2. Methodology

In this section, the predominant extraction procedures of existing SOPs are highlighted in stepwise fashion. Novel approaches are introduced to increase the effectiveness and decrease the potential artifacts of specific steps, while also taking into consideration current concerns in the toxicology community.

2.1. Ultra-sonication

The underlying methodology for most filter extraction techniques, including those used in the current study and the EPA SOP, involves sonication followed by lyophilization (*personal communication*, Bowser, 2009; Devlin, 2009). Sonication – where ultrasonic energy is applied to a liquid to nucleate, grow and implosively collapse microscopic bubbles – is required to remove the PM from the filter media and suspend or dissolve it in solution, typically high-purity water. Sonication is necessary because of the adhesive and cohesive nature of filter substrates and most fine and ultrafine PM, the high impaction velocity of the PM onto the substrate during sampling, as well as the fairly ubiquitous presence of hydrophobic PM components. Ironically, the very properties of filter substrates responsible for ultrahigh, and thus desirable, PM retention efficiencies during sampling also make it difficult to extract that PM post-collection. It is the cavitation energy of the imploding microscopic bubbles during sonication that actually breaks the adhesive/cohesive forces holding the particles together and to the filter media.

In fact, the cavitation energy is sufficient to degrade the integrity of the filter to the point that microscopic pieces are broken off and further fragmented into micron-sized PM, thus contaminating the sample. This extraction artifact has recently garnered much attention in the toxicology community (*personal communication*, Pinkerton, 2010; Tablin, 2011; Van Winkle, 2012; Wilson, 2011). Although filter materials – e.g. glass microfibers, Teflon and polyurethane foam (PUF) – are generally considered to be chemically inert in terms of eliciting toxicological effects, there is concern about the effects of the size and morphology of the fragmented filter particles (FFP) on the respiratory tract of the animals used for in vivo studies and the cell cultures employed in in vitro studies. This is especially true for traditional afterfilters like the Pallflex® line of Tissuquartz™, Fiberfilm™ and Emfab™ filters that are composed of glass microfibers to enhance the retention of organic

PM components. There is evidence that sonication-derived, micron-sized, needle-like glass fibers can have a pronounced effect on cell viability when administered to cell cultures (*personal communication*, Tablin, 2011). As a result, a significant amount of attention has been given in the current study to separating FFP from the extracted PM.

The approach chosen here takes advantage of the size difference between sonication-derived FFP and the extracted PM to selectively filter the former from the sonication solution using a porous filter membrane of known porosity that retains the FFP but allows the PM particles to pass through into the filtrate. The idea stems from the fact that mechanical abrasion based particle formation, like the production of FFP via sonication, will tend to produce a distribution of particle sizes – typically lognormal in nature – where the mean of that distribution resides high in the supermicron range. Therefore, ambient submicron PM samples should be more than an order of magnitude smaller than the FFP, allowing separation. There are three caveats though:

1. Particles have a tendency to agglomerate on the filter during sampling and although sonication does a good job fragmenting these agglomerates and dispersing the particles into solution, it cannot completely restore the original size distribution of sampled PM and thus a small fraction of particularly cohesive PM may be retained by the porous membrane.
2. Some PM may agglomerate with the FFP and thus be retained during filtration.
3. The spread in the size distribution of FFP is generally unknown but it is likely that the leading tail dips down into the micron and submicron ranges. Any FFP smaller than the membrane pore size will likely pass through into the filtrate and contaminate the sample. This is evidenced by the fact that a small amount of filter material is still recovered even when selective filtration is applied to the sonication extract from clean filter blanks using pore sizes less than a micron, as will be shown later.

Membrane filters are available in a variety of pore sizes and the objective is choosing the pore size that maximizes FFP removal and minimizes particle loses. In the current study, the pore size was 8 μm since *(a)* smaller pore sizes did not measurably decrease the mass of FFP recovered from filter blank extractions, *(b)* this pore size is an order of magnitude larger than the particle size fractions studied and about a factor of three larger than the $PM_{2.5}$ size cut used in most ambient PM toxicology studies, and *(c)* there was no evidence for retention of agglomerated PM. In this study, the maximum size of the PM was 1 micron so there was substantial size separation between the PM of interest and the size distribution of the FPP. In studies where larger PM sizes are being studied, further consideration must be given to the appropriate pore size.

Sonication in water does a fairly good job of removing a majority of PM from the afterfilter, especially water soluble components. Extraction efficiencies on the order of 70% are commonly reported using this method alone (*personal communication*, Devlin, 2009). However, there is concern about what is not removed, i.e. the other 30%. These PM components are likely water insoluble and/or exhibit abnormally strong cohesive binding to the filter fibers, and may be lipid soluble so possibly eliciting health effects. The exclusion of these components introduces a compositional bias in the extracted PM relative to what was sampled from the atmosphere. To address these issues, afterfilters were sequentially sonicated in different solvents of varying polarity, including water (H_2O; polar protic), dichloromethane, (DCM; polar aprotic) and hexane (Hx; non-polar); the choice of the latter two will be discussed in more detail later. After the initial water sonication, the PM deposit is still clearly visible against the white background of the afterfilters and the additional organic solvent sonications do a good job of removing a portion of the remaining material, as evidenced in both the mass of PM recovered and reduced contrast between the deposits and filter material. For example, as shown later, the average percent of the total extracted PM mass recovered by organic (DCM and Hx) solvent sonication for all afterfilters used in this study is $20 \pm 10\%$. As with the water sonication extract, the solvent sonication extracts are filtered to remove any FFP and then added back to the dry PM recovered from lyophilization, as discussed in the next section. Since organic solvents will partially dissolve PUF substrates, leaving a contaminant residue in the sample, organic solvent sonication was only possible for the UF PM collected on afterfilters. For example, during the testing stages of protocol development, an average of 6.0 ± 0.06 mg of PUF material was removed by solvent sonication of cleaned PUF substrates, whereas the total PM mass on such substrates may be as little as 1 mg or so.

2.2. Lyophilization

Lyophilization (or freeze drying) is used to recover dry PM after sonication by removing the water and is necessary to accurately determine the mass of extracted PM for toxicological studies, which rely on accurate dose mass. During lyophilization, the sonication solution – comprising extracted PM dissolved and suspended in water – is frozen to a very low temperature, typically on the order of −80 °C, and then subjected to high vacuum (~0.1 mbar) to sublimate the ice, leaving behind dry PM. Initially, the PM is completely encased in ice and protected from vacuum conditions but as the ice recedes and the particles are exposed, there is concern that a significant amount of material may volatilize from the PM. In fact, during the final stages of lyophilization the PM is subjected to high vacuum for extended periods while the last remaining amounts of ice are sublimated. There is no doubt that some fraction of semi-volatile primary and secondary PM components, and even nonvolatile organics, will be lost during this process. For example, results from this study show that as much as 20–40% of the solvent extractable organics can be lost during lyophilization, as discussed in a following section. Given the importance of the organic PM fraction in terms of toxicological testing, this is an artifact that was given significant attention when developing the filter extraction techniques deployed in the current study. To circumvent this artifact, liquid–liquid extractions using various chemical solvents were performed to remove the organics prior to lyophilization, which were then added back into the dry PM afterward. Furthermore, during the final stages of lyophilization dry particles can be entrained into the flow of water vapors exiting the lyophilization flask so it is necessary to insert a filter between the flask and lyophilization chamber to achieve 100% recovery of the dry PM.

Numerous organic solvents with varying properties are available but for the purposes of the current study, selection criteria included: *(a)* Since the organics are being removed from particles suspended in water after sonication, the solvent must be immiscible with water so that the two can be separated from each other after the liquid–liquid extraction. Therefore, only select non-polar and polar aprotic solvents can be used. *(b)* The solvent must have a very high vapor pressure so that it can be evaporated quickly and thoroughly under mild environmental conditions with minimal evaporation of the extracted organic compounds. *(c)* The solvent must act as a universally strong solvent to maximize the number of organic compounds solvated and be unreactive to ensure that extracts are chemically unaltered. Given these requirements, and in attempts to cover the polarity range of organic compounds, DCM

and Hx were chosen for the current study. DCM and Hx are also commonly used in the filter extraction step of sample preparation protocols for GC–MS analysis of particulate organic carbon for molecular speciation (Fine et al., 2001, 2004; Ham and Kleeman, 2011; Schauer et al., 1996, 1999; Sheesley et al., 2004). Other organic solvents, such as acetone, are used in the filter extraction step of sample preparation for trace element analysis via ICP-MS (Herner et al., 2006). Acetone is miscible with water so is inappropriate here but these studies suggest that organic solvent extraction is necessary for trace element and molecular analyses of most combustion generated aerosol and/or secondary organic aerosol since *(a)* the trace metals are typically encapsulated by layers of organic compounds and *(b)* most organic compounds are hydrophobic and thus are not likely removed from the filter to any significant degree by water alone.

In the current study, DCM and Hx were sequentially added to the particle laden water from the sonication step in a separatory funnel and shaken vigorously. The layers are separated and the solvents evaporated under a nitrogen (N_2) atmosphere to recover the solvent soluble fractions. Some fraction of the water soluble organic compounds are likely removed during this process since DCM is polar, but it is not clear how much and for which compounds. The remaining water solution is lyophilized and the solvent soluble fractions are then added back to the dry PM from lyophilization. The organic solvent sonication extracts, discussed previously, are handled in the same manner; solvents are evaporated under a N_2 atmosphere and the recovered PM added to everything else. Detailed, step-by-step descriptions of the full extraction protocols, as well as flow diagrams, for the afterfilters and PUF substrates used in the current work are given in the Supplementary material.

2.3. Gravimetric analysis

Gravimetric analysis – comprised of pre- and post-weighing filters, beakers and storage vials – is one of the most challenging parts of the entire filter extraction process but also one of the most important steps in terms of quantifying dose response and normalizing all extracts to equal mass doses for toxicological studies. There are numerous reasons for this difficulty but the underlying fundamental challenge is subtracting two relatively large masses to obtain a very small mass. For instance, filters, beakers and storage vials weigh on the order of tens of grams, whereas PM component masses at various stages of the filter extraction process weigh on the order of tens to hundreds of micrograms – a 5 orders of magnitude difference. As a result, measurement errors are large and these errors are compounded as they propagate through the various calculations of the analyses.

Random errors associated with the actual measurements are modest (on the order of tens of micrograms) and fairly easy to quantify by weighing the same object multiple times to obtain the average and standard deviation in the measurement, which can be used to define the confidence interval. Confidence intervals are then propagated through the calculations to obtain an error estimate for the calculated values. Systematic errors due to the effects of day-to-day fluctuations in environmental variables on both the balance and mass of an object, however, can be substantially larger (on the order of milligrams in some cases) and harder to track. The most important environmental variables are (*a*) temperature and relative humidity, which can affect the mass of an object (e.g. filters and PM), (*b*) the mass of the air column above the balance and the mass of any air within the object relative to the surrounding air (e.g. buoyancy effects for beakers and storage vials), (*c*) background electromagnetic radiation, which directly affects the operation of the balance and the concentration of charged particles in the air, (*d*) static charge, which alters the mass of an object by affecting its interaction with the surrounding environment and the operation of the balance, and (*e*) in the case of storage vials, the amount of time under vacuum during the lyophilization process.

The latter phenomenon deals with the adsorption of gas phase molecules on the surfaces of the storage vials when they are exposed to ambient lab air for extended periods, and the desorption of these molecules during the lyophilization process when the vials are subjected to vacuum. All storage vials are weighed under vacuum since, for reasons stated above, it is substantially more robust and precise compared to weighing vials with their contents at ambient conditions; the mass of air in a 10 mL vial at STP is ~17 mg.

The difference in the weight of a given vial under vacuum can vary between zero and more than a milligram depending on the difference in time the vial was put under vacuum prior to weighing. Prior to pre-weighing, vials are attached to the lyophilizer and pumped down to operating conditions (~0.1 mbar), which takes less than a minute, before being sealed, removed and weighed. This process can be repeated with a high degree of precision in the measured masses. During lyophilization of an actual extracted PM sample, the vial remains under vacuum for periods on the order of several hours and the actual mass of the vial decreases due to this desorption phenomenon. Therefore, using the difference between pre- and post-weights of vials in these situations creates a systematic error in calculated PM masses.

The best way to track and quantify these errors, as well as the other systematic errors listed above, has been to incorporate standard reference vials and beakers that are subjected to the exact same procedures and conditions as the sample vials and beakers but never have anything added to them. Tracking differences in the measured masses of these standards throughout the entire filter extraction process provides a quantitative metric of systematic error that can be used to correct the PM mass calculations. This has been done for all of the calculations presented here. For example, the average mass lost by standard reference vials during lyophilization was 0.7 ± 0.2 mg and the variation in beaker mass was on the order of ±30 μg. Beakers are not weighed under vacuum so errors are largely due to static charge and buoyancy effects.

Finally, pre- and post-weighing afterfilters and PUF substrates was extremely challenging and highly unreliable throughout this entire work. This is due to a combination of factors, namely *(a)* the size of the filters relative to the balance weighing pan (afterfilters are 6.75″ in diameter and PUF substrates are annular rings with an outer diameter on the order of 5″ while the weighing pan is only 3.1″ in diameter), *(b)* the effects of static charge, especially for the PUF, and *(c)* the effects of relative humidity on the absorption of water by both the filter substrate and deposited PM.

3. Results and discussion

The filter extraction techniques described above have been applied to all of the afterfilters and PUF substrates used during both the S08 and W09 source-oriented sampling experiments described previously. This includes the UF and SMF size fractions of nine separate CVs for S08 and 10 separate CVs for W09, as well as afterfilter and PUF field blanks. All afterfilters and PUF were precleaned prior to sampling via successive sonications in Milli-Q H_2O; afterfilters were also solvent washed with ethanol. In all of the discussion and figures that follow, ChemVols are referenced by their source assignment – determined by extensive source attribution efforts published elsewhere (Bein et al., submitted for publication) – rather than the arbitrary numbering system employed in prior work (Bein et al., 2009).

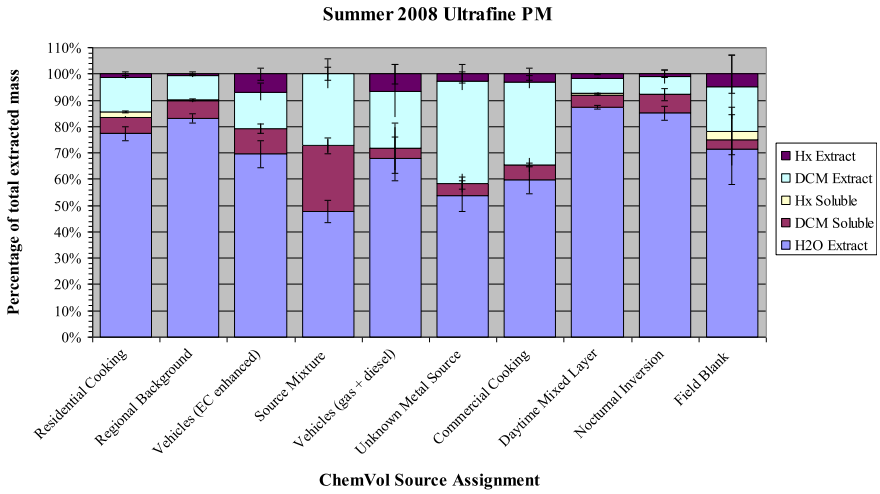

Fig. 1. The fractional distribution of total extracted mass among the PM components removed during the various steps of the filter extraction process for the ultrafine PM fractions collected during summer 2008 by ChemVol; see text for discussion.

3.1. PM component distributions

Figs. 1 and 2 show the UF fractional distribution of total extracted mass among the PM components removed during the various steps of the filter extraction process during S08 and W09, respectively; Figs. 3 and 4 correspond to the associated SMF fractions. Results from the exact same procedures applied to afterfilter and PUF field blanks are also included. The relevant PM components common to both the afterfilter and PUF extractions are *(a)* filtered and liquid–liquid extracted H_2O sonication extracts (**H_2O Extract**) and *(b)* DCM and Hx soluble fractions removed by liquid–liquid extraction of the H_2O sonication solutions (**DCM Soluble** and **Hx Soluble**). The sum of these fractions represents the total PM recovered using existing SOPs, with the exception of one consideration discussed in the next section. The additional steps introduced in the new methodology include *(a)* filtered DCM and Hx sonication extracts (**DCM Extract** and **Hx Extract**) for the afterfilters and *(b)* filtered and liquid–liquid extracted H_2O sonication extracts of mechanically chopped (MC) PUF substrates (**MC H_2O Extract**) and DCM and Hx soluble fractions removed by liquid–liquid extraction of the MC H_2O sonication solutions (**MC DCM Soluble**) and (**MC Hx Soluble**). The sum of these additional fractions represents an increase in the extracted PM mass, and thus an increase in the extraction efficiency, achieved by the new methods relative to existing SOPs.

The most interesting thing to note in these figures is that the fraction of total extracted mass recovered during each step of the extraction process is a function of source or source mixture such that extraction efficiencies are source specific. This makes sense given that different sources emit particles containing different components and that these PM components will be removed more or less efficiently by each of the extraction steps. In other words, the efficiency of different extraction steps is compositionally dependent such that omission of any step represents a potential compositional bias in the reconstituted PM. A comprehensive reconciliation of the fractionation of extracted PM mass based on the ChemVol source assignments and single particle compositional signatures used to make those assignments depends on many complicated factors and thus will not be attempted here.

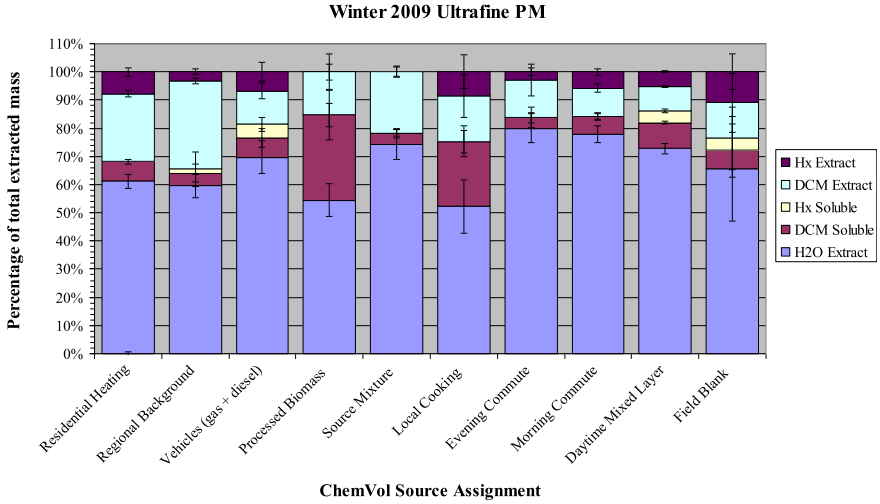

Fig. 2. The fractional distribution of total extracted mass among the PM components removed during the various steps of the filter extraction process for the ultrafine PM fractions collected during winter 2009 by ChemVol; see text for discussion.

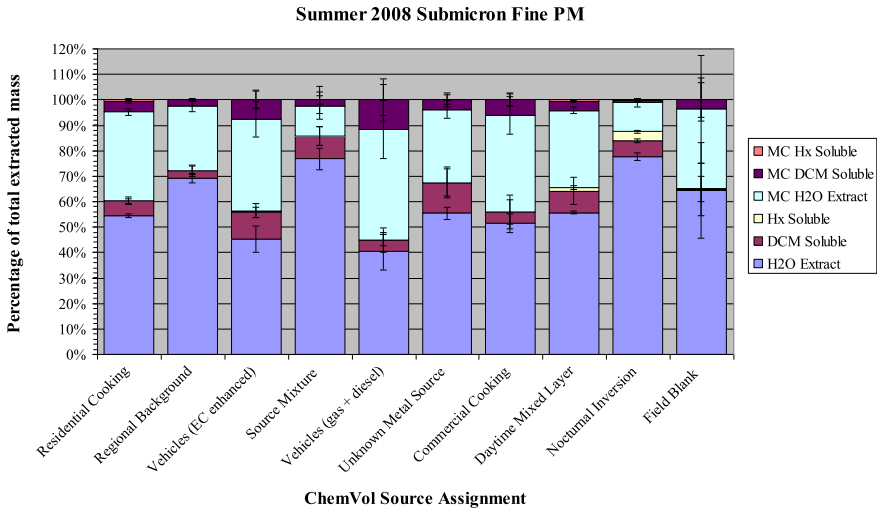

Fig. 3. The fractional distribution of total extracted mass among the PM components removed during the various steps of the filter extraction process for the submicron fine PM fractions collected during summer 2008 by ChemVol; see text for discussion.

3.2. Extraction efficiencies

Extraction efficiency is defined as the fraction of the total PM mass sampled that is removed during the filter extraction process. Direct calculation of extraction efficiencies was not possible in this work due to various problems encountered during pre- and post-weighing filters, as described previously, resulting in unreasonable, inconsistent and error prone calculations. Furthermore, filters were sectioned and extractions performed on these sections rather than on whole filters, further reducing confidence in total PM mass calculations. However, the point of this work is to show increased efficiency, decreased compositional bias and decreased artifacts for the new methods relative to existing SOPs and the study was designed to demonstrate this. Given the paucity of extraction efficiencies in the literature, especially for source-oriented samples, direct inter-comparison of extraction efficiencies between methods is not possible. Instead, as mentioned previously, the conventional H_2O sonication and lyophilization employed by existing SOPs – comprising the **H_2O Ex** fraction and the **DCM Soluble** and **Hx Soluble** fractions – was included as the first step in the new methods so that the increase in extraction efficiency gained by the additional steps could be directly calculated as a function of source or source mixture. The remaining fraction of total extracted mass – i.e. the **DCM Ex** and **Hx Ex** fractions from the afterfilter extractions and the **MC H_2O Ex**, **MC DCM Soluble** and **MC Hx Soluble** fractions from the PUF extractions – represents an increase in extraction efficiency over existing SOPs.

Another important consideration here is that a portion of the **DCM soluble** and **Hx Soluble** fractions recovered during liquid–liquid extraction of the afterfilter and PUF water sonication solutions will be lost during lyophilization due to evaporation. Although this could not be determined for all samples given the limited availability, and thus high value, of source-oriented PM, it was determined for the UF and SMF fractions of the **Daytime Mixed Layer** and **Nocturnal Inversion** CVs since these CVs were operated daily for extended periods during timed intervals and thus collected significant amounts of mass. The percent loss of the combined DCM and Hx soluble fractions from these CVs during lyophilization ranged from 15 to 50% with an average of 33 ± 19% over the 16 different tests performed. Using this value to adjust the (**DCM Soluble** + **Hx Soluble**) fractions and then adding the (**DCM Ex** + **Hx Ex**) and (**MC H_2O Ex** + **MC DCM Soluble** + **MC Hx Soluble**)

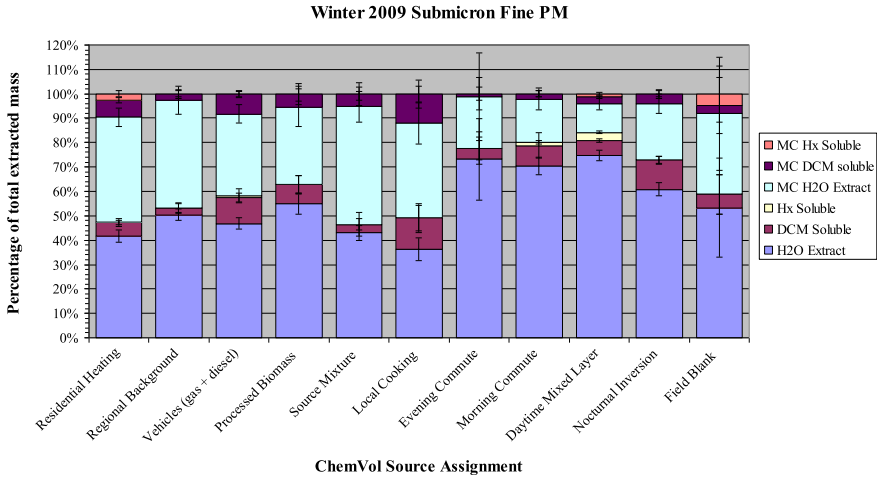

Fig. 4. The fractional distribution of total extracted mass among the PM components removed during the various steps of the filter extraction process for the submicron fine PM fractions collected during winter 2009 by ChemVol; see text for discussion.

fractions from the afterfilter and PUF extractions, respectively, the percent increase in extraction efficiency of the new methods relative to existing SOPs as a function of source or source mixture and size fraction is shown in Fig. 5 for both the S08 and W09 experiments.

On average, conventional methods from existing SOPs only account for roughly 75% and 60% of the total extracted UF and SMF PM mass, respectively, obtained using the new methods described here. Equally important is that these increases in extraction efficiency for the new methods (~25%/40% for UF/SMF) are compositionally specific, as evidenced by the fact that additional organic solvents were required to obtain them, suggesting that the new methods do a better job of conserving bulk chemical composition during extraction and that existing SOPs may be compositionally biased, especially in the less polar organic components that may be lipid soluble.

In terms of the filter blank extractions, the average mass of FFP recovered during a single water sonication without any subsequent Millipore membrane filtration was 1.8 ± 0.2 mg and 0.8 ± 0.2 mg for the afterfilters and PUF, respectively, as determined from six separate measurements. This should be representative of the relative amount of contaminant FFP incorporated into extracted PM samples using existing, conventional SOPs. The average mass of FFP recovered after full application of the new extraction protocols, as determined from triplicate measurements, was 0.64 ± 0.06 mg and 0.55 ± 0.04 mg for the afterfilter and PUF field blanks, respectively, representing an overall percent reduction in FFP over conventional methods of 66 ± 5% and 30 ± 10%, respectively. The fraction of this FFP recovered after Millipore membrane filtration of the water sonication extracts, as well as the Buchner funnel filtrations of the solvent washes, was 0.8 ± 0.1 and 0.6 ± 0.1, respectively, while the remaining FFP can be attributed to the additional organic solvent and mechanically chopped sonications. Therefore, the average removal efficiencies of the various filtration steps employed in the new extraction protocols are 73 ± 6% and 60 ± 10% for the afterfilter and PUF filter blanks, respectively. This represents a significant reduction in contaminant FFP when using the new extraction protocols compared to existing SOPs. An important caveat to note here, however, is that the amount and size of FFP formed during extraction is likely proportional to the filter surface area exposed during sonication. Since significantly more filter surface area is exposed during sonication of filter blanks compared to filters loaded with PM, a direct correlation may not exist between the FFP recovered from filter blanks and that truly contaminating extracted PM samples so the filter blank results may be misleadingly high. Unfortunately, there was no way to quantify this during the current study and the full field blank extractions, for lack of a better approach, were used as a control in the toxicological studies. Another important thing to note is that the afterfilters and PUF were pre-cleaned via sonication, as mentioned previously, significantly reducing the amount of FFP recovered during the field blank extractions relative to no sonication pretreatment.

An added benefit of the new methodologies presented here is the ability to provide toxicologists with fractionated PM samples to test the differential toxicity of certain PM fractions, or components; e.g. water extractable components versus organic solvent extractable components or differences between solvent-specific extracts. Studies of diesel exhaust particles have shown that different PM components elicit different responses from a given toxicological assay and, more interestingly, that the sum of responses to individual PM components is significantly larger than the response to the composite of those components (DeMarini et al., 2004; Hayakawa et al., 1997). In other words, the presence of assay-specific toxicologically inert PM components may interfere with the response to the toxicologically active PM components.

3.3. Method mass closure

For the filter extraction techniques employed in this study, method mass closure was assessed by comparing the total extracted PM mass obtained from weighing the reconstituted composite extracts in the final storage vials to that obtained by summing the

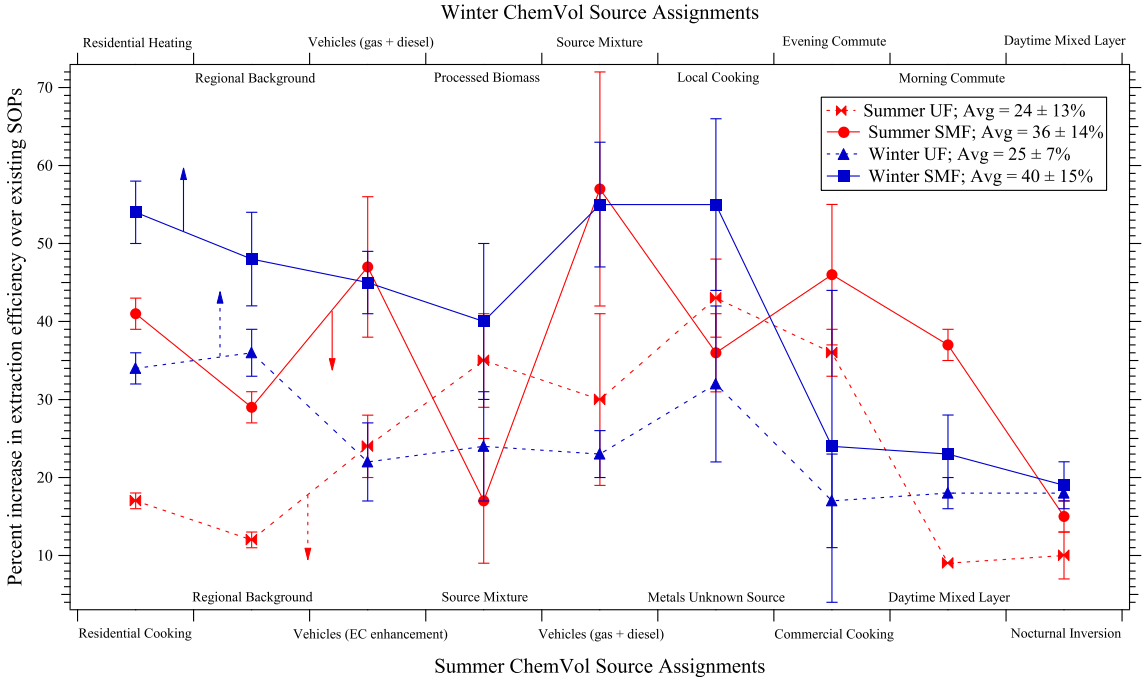

Fig. 5. Percent increase in extraction efficiency for the new methods relative to existing SOPs as a function of source or source mixture and size fraction for the S08 and W09 experiments; see text for a discussion on how these values were calculated.

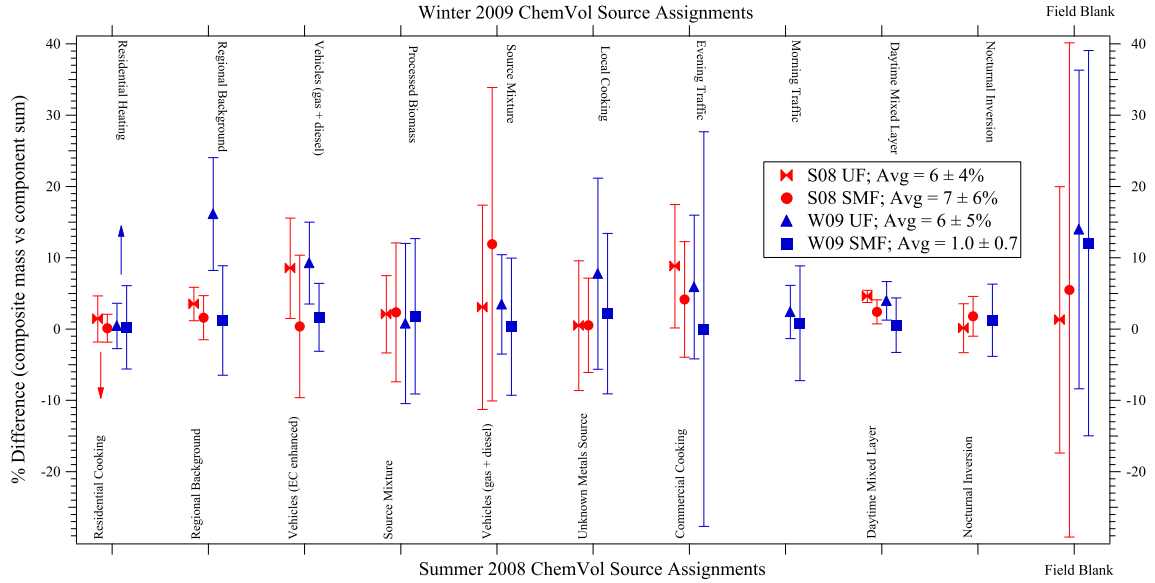

Fig. 6. Percent difference between the total extracted mass obtained by weighing the final reconstituted composite extracts versus summing the component masses obtained during the various steps of the filter extraction process; see text for discussion.

masses of the individual PM components extracted during the various steps of the filter extraction process; i.e. **H_2O Ex** + **DCM Soluble** + **Hx Soluble** + **DCM Ex** + **Hx Ex** for the afterfilters and **H_2O Ex** + **DCM Soluble** + **Hx Soluble** + **MC H_2O Ex** + **MC DCM Soluble** + **MC Hx Soluble** for the PUF. These data are shown in Fig. 6 as the percent difference between the composite mass and component sum by ChemVol, size fraction and sampling campaign; values are listed in Tables S1 and S2 of the Supplementary material. Results from the extraction of afterfilter and PUF field blanks are also included. Note that this analysis is not a mass closure in terms of the total PM mass sampled but rather demonstrates total extracted PM mass closure via the sum of its components, which is an important method validation exercise given the novelty and number of steps included. The average percent difference over all filter extractions performed during this study was only 4%, demonstrating good closure given the many potential sources of uncertainty and error.

4. Conclusions

The underlying impetus of this work was not only a specific need to develop methods that maximize extraction efficiencies while minimizing compositional biases and extraction artifacts but also by a general paucity of existing literature on the subject. Amazingly, and to the authors' knowledge, there has never been a systematic study published in the literature that comparatively investigates the various filter extraction SOPs in terms of the relevant parameters, despite the importance of these methods to toxicological studies. The single most important result of this study is that significant differences in the physical and chemical properties of extracted PM can exist depending on the extraction method deployed and that this further depends on the source or source mixture contributing to the PM being extracted. Since the primary purpose of many filter extractions is for subsequent toxicological testing, differences in material extracted from the filter could potentially result in differences in toxicological response for a given PM sample; i.e. toxicological outcomes may be partially or wholly dependent on the extraction SOP employed. This could be a potential source of seriously misleading information for the various stakeholders, including government agencies and policy makers, responsible for protecting human health.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atmosenv.2014.03.042.

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