Atmospheric Environment 83 (2014) 35–42

Contents lists available at ScienceDirect

Atmospheric Environment

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

Measurement of the oxidative potential of $PM_{2.5}$ and its constituents: The effect of extraction solvent and filter type

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HIGHLIGHTS

- We examined effects of filter type and extraction solvent on oxidative potential (OP).
- Extraction solvent had a significant effect on OP^{DTT}, but not on OP^{ESR} or OP^{AA}.
- OP values measured from quartz filter extracts were heavily attenuated for all assays.
- However, OP values from quartz filters were highly correlated with those from Teflon.
- OP measured with ESR direct method showed promising results.

ARTICLE INFO

Article history:
Received 11 June 2013
Received in revised form
25 September 2013
Accepted 23 October 2013

Keywords:
Oxidative potential
DTT
ESR
Ascorbate acid depletion
Reactive oxygen species
Particulate matter

ABSTRACT

The capacity of Particulate Matter (PM) to oxidise target molecules, defined as its oxidative potential (OP), has been proposed as a biologically more relevant metric than PM mass. Different assays exist for measuring OP and their methodologies vary in the choice of extraction solvent and filter type. Little is known about the impact of extraction and filter type on reported OP. Four a-cellular assays; electron spin resonance (ESR), dithiothreitol (DTT), ascorbate acid depletion (AA) and reductive acridinium triggering (CRAT) assay were chosen to evaluate whether these differences affect the OP measurement, the correlation between OP from different assays and the association with PM chemical composition. We analysed 15 urban 48–72 h $PM_{2.5}$ samples collected on quartz and Teflon filters. The choice of extraction solvent had only a significant effect on OP^{DTT}, while all OP measures for quartz filters were heavily attenuated. OP values derived from quartz were, however, highly correlated with those derived from Teflon. OP^{DTT} correlated highly with OP^{CRAT}, and OP^{ESR} correlated highly with OP^{AA}. These correlations were affected by the choice of filter type. Correlations between OP and PM chemical composition were not affected by filter type and extraction solvent. These findings indicate that the measurement of relative OP reactivity is not greatly influenced by filter type and extraction solvent for the investigated assays. This robustness is also promising for exploratory use in monitoring and subsequent epidemiological studies.

1. Introduction

It has been recognized that increased exposure to ambient particulate matter (PM) is associated with a wide range of adverse health effects (Brunekreef and Holgate, 2002; Pope III and Dockery, 2006). Currently, PM is regulated based on mass concentration, whereas evidences indicate that the chemical composition, surface area and other characteristics of PM are more closely linked to the

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1352-2310
http://dx.doi.org/10.1016/j.atmosenv.2013.10.049

induction of toxic responses (Nel, 2005). Oxidative stress, initiated by the presence and formation of reactive oxygen species (ROS), or free radicals, has been considered an important mechanism to the particle-induced health effects (Delfino et al., 2005). Although the human body is capable of dealing with ROS, diseases can overwhelm or impair this host defence mechanism (Delfino et al., 2011). In that case, ROS can trigger a cascade of events eventually leading to for example airway and pulmonary inflammation. This in turn can cause a range of adverse effects like cell and tissue damage (Nel, 2005). Oxidative potential (OP) is defined as a measure of the capacity of PM to oxidise target molecules, i.e. by generating ROS in environments without living cells. It has been proposed as a metric that is better related to biological responses to PM exposures and thus could be more informative than mass alone (Borm et al., 2007). Several methods for testing OP have been developed, but no consensus has been reached yet as to which assay is most appropriate (Ayres et al., 2008). Furthermore, few inter-assay comparisons have been published.

Various assays exist to assess the oxidative capacity of PM, each with a different sensitivity to the ROS generating compounds. Electron spin resonance (ESR) with 5,5-dimethylpyrroline-N-oxide (DMPO) as a spin trap, measures the ability of PM to induce hydroxyl radicals (•OH) (Shi et al., 2003a, 2003b) in the presence of H_2O_2. The consumption of dithiothreitol (DTT) is based on the ability of redox active compounds to transfer electrons from DTT to oxygen (Cho et al., 2005; Kumagai et al., 2002). Other common assays involve measuring the ability of PM to deplete antioxidants such as vitamin C, glutathione and uric acid (Mudway et al., 2004). Due to high detection sensitivity, fluorescent-based probes have also been used to quantify PM-related ROS. These are based on the principle that a fluorescent product is generated when the non-fluorescent probe molecule reacts with ROS. The most common used probe is 2,7-dichlorofluorescein (DCFH) (Landreman et al., 2008). Another system with high sensitivity consists of chemiluminogenic compounds, where certain acridinium esters, e.g. 4-methoxyphenyl-10-methylacridinium-9-carboxylate, have a high selectivity for superoxide (Yamaguchi et al., 2010). Using this principle, a ROS assay using acridinium esters (CRAT) as a redox probe was developed (Zomer et al., 2011).

PM is usually collected on filters. After sampling, various methods are used to extract the PM from the filters into suspension and then used for physical, biological, chemical and toxicological analyses. The choice of extraction solvent varies between laboratories ranging from deionised water to organic solvents (e.g. dichloromethane, methanol). This has an effect on the efficiency of which PM species will be extracted and, as such, their toxicological properties (Eiguren-Fernandez et al., 2010; Verma et al., 2012). Polytetrafluoroethylene (PTFE), also known as Teflon, glass and quartz are frequently used PM sampling filter types. Quartz is often used when PM is sampled for composition analysis. In the literature, most a-cellular assays for assessment of oxidative potential assessment have been applied to Teflon filters and information on the usage of quartz filters is limited. Presently, quartz filters are used as reference material within the PM10 standard (EN 12341) in the national air monitoring networks in EU, while quartz, glass fibre, PTFE and PTFE-coated glass fibre are allowed within the $PM_{2.5}$ standard (EN 14907) ("http://ec.europa.eu/environment/air/quality/legislation/assessment.htm", 2012).

In the framework of the OPERA project (Oxidative Potential Exposure and Risk Assessment); we aim to evaluate the value of OP as a health relevant PM metric for air quality assessment and regulation. Given the different filter types and extraction methods, our primary goal for this study was to assess if the aforementioned differences in methodologies might affect the measurement of OP. Additionally, we aimed to see how this influenced the correlation between OP from different assays and the association with PM composition. We evaluated this for four a-cellular OP assays. ESR, depletion of ascorbate assay and the reductive acridinium triggering (CRAT) assay.

2. Materials and method

2.1. Study design

Ambient $PM_{2.5}$ samples were collected at two locations in the Netherlands using Harvard Impactors operating at 10 L min^{-1} flow (Air Diagnostics and Engineering Inc., Naples, Maine, USA). The sampling sites were located at an urban background site in Rotterdam and along a busy highway in Amsterdam. The sample volume was calculated using elapsed time indicators and flow readings. The sampling periods were five (day 1–5) 48–72-h measurements in Rotterdam and ten (day 6–15) 48–72-h measurements in Amsterdam from August to November 2011. Five collocated pump units, each containing four Harvard Impactors of which two used 37-mm Teflon (2 μm pore size, PVC support ring, Pall Corp., NY, USA) and two 37-mm quartz filters (prebaked, QMA, Whatman - GE Healthcare Biosciences Corp) were operated simultaneously. Thus, ten Teflon filters and ten quartz filters were collected for each measurement period.

To determine particle mass concentrations, filters were weighed before and after sampling, in accordance to EN 14907, in a climatised room at an average temperature of 20 ± 1 °C and 50 ± 5% relative humidity using a microbalance (Model MT5, Mettler–Toledo Ltd., Greifensee, Switzerland) with 1 μg precision. Until processing, the filters were stored in petri dishes at 4 °C in the dark. The OP analyses were completed by September 2012.

2.2. Extraction procedures

In our experiments, the Teflon filters were extracted with methanol (HPLC grade, Biosolve BV, Valkenswaard, Netherlands) and TraceSELECT ultrapure water (Sigma, Zwijndrecht, Netherlands). The quartz filters were only extracted with methanol, and additionally analysed directly on filter with ESR. See Supplement information (SI) Table S 1 for an overview of how filter duplicates were allocated to different extraction procedure and OP analysis.

2.2.1. Methanol extraction

The filters were immersed with methanol in a petri dish and extracted in an ultrasonic bath (Branson 5510 Ultrasonic cleaner, 40 kHz). The extract was then transferred to a rounded glass flask and reduced in volume using the evaporator set (RV 10 Basic Rotary Evaporators, IKA Works, VWR, USA) at 30 °C until about 1 mL was left. The filtrates were then transferred to Eppendorf vials and dried overnight at 30 °C under a constant flow of nitrogen. The quartz filter extracts were filtered through a 0.45 μm PTFE syringe filter (VWR, Breda, Netherlands), to remove the high amount of observed quartz fibres.

2.2.2. Water extraction

Water based extractions were only performed on Teflon filters using the method by Shi et al. (Shi et al., 2003b). Briefly, the filters were immersed in 2 mL deionised water followed by five minutes shaking, five minutes sonication (Bandelin Sonorex RK-52, 60/120 KW, 35 KHz) in a sonication water bath and finally five minutes vortexing (rpm 2800).

We attempted water extraction on the quartz filters, but it was proven difficult as the filters readily absorbed water, thus requiring large quantities of water (>5 mL). Due to the low mass loading (79–

1000 μg of PM) on several filters, adding such a substantial amount of water might result in too heavily diluted suspensions. This is without taking into consideration loss due to extraction. In addition, sonication destroyed the quartz fibres making it difficult to separate quartz fibres from particles when filtering the suspensions.

2.3. Elemental composition analysis

Teflon filter duplicates for each sampling day, and two lab blanks were analysed with energy dispersive X-ray fluorescence spectrometry (ED-XRF) at Cooper Environmental Services (Portland, OR, USA) to get the elemental composition. Not enough quartz filters were obtained due to pump failure, thus additional composition analysis was not possible. All elements of interest (see SI Table S 3) were above detection limit (LOD) in all samples except for aluminium (Al) and nickel (Ni) (1–3 samples < LOD). The limit of detection was calculated as 3 times the standard deviation of the lab blanks. The coefficients of variation (CV) values, as a measure for the precision of duplicate measurements, were less than 25%. CV values were calculated as the sum of the squared absolute differences of the duplicates, divided by two times the number of duplicates. The square root of this value was then divided by the mean and multiplied by 100 to get the percentage (Eeftens et al., 2012).

Carbon analysis (EC/OC) was performed on quartz filers using a Thermal Optical Transmittance (TOT) analyser (Sunset Laboratory Inc., Tigard, OR, USA). From each filter, a punched sample of 1.5 cm^2 was analysed with the temperature protocol EUSAAR_2; a standard protocol developed for European aerosols (Cavalli et al., 2010). Optical transmittance is used to correct for charring. For quality assurance, we analysed every 4th filter twice.

2.4. Oxidative potential assays

2.4.1. DTT

The DTT assay measures the presence of reactive oxygen species via formation of the DTT-disulfide due to transfer electrons from DTT to ROS by recycling chemicals such as quinones (Cho et al., 2005). Typically compounds which react in this assay are organic species (e.g. quinones), but previous studies have shown that transition metals can also oxidize DTT (Charrier and Anastasio, 2012; Lin and Yu, 2011).

In brief, aliquots of $PM_{2.5}$ water-reconstituted methanol and water extracts were incubated with DTT (Sigma, Zwijndrecht). The reaction was stopped at designated time points (0, 10, 20, 30 min), adding 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) (Sigma, Zwijndrecht). The absorbance at 412 nm is recorded, and the rates are calculated using linear regression of the data as seen from a plot of absorbance against time. The results are expressed as nmol DTT min^{-1} per sampled volume or per μg of PM. Domestic oil burning furnace (DOFA, obtained from US EPA, RTP, NC) with a fixed concentration was used as a positive control and ultrapure water as a negative control.

2.4.2. Ascorbate (AA) depletion assay

The AA assay is a simplified version of the synthetic respiratory tract lining fluid (RTFL) assay (Zielinski et al., 1999), where only ascorbate acid is used. This assay can be used to quantify the transition metal-based redox activities, but has also shown to be sensitive to quinones (Roginsky et al., 1999).

AA analysis was performed according to the protocol by Mudway et al. (2011), but TraceSELECT ultrapure water was used instead of Chelex-resin treated water. Briefly, PM extracts are incubated in the spectrophotometer (spectraMAX 190: Molecular Devices, Sunnyvale, USA) for 10 min at 37 °C. After adding ascorbate acid, the absorption at 265 nm is measured every 2 min for 2 h. The 96-wells plate is auto shaken for 3 s before each measurement. The maximum depletion rate of ascorbic acid is determined by linear regression of the linear section data, plotted as absorbance against time. The results are expressed as nmol s^{-1} of max AA depletion per sampled volume or per μg of PM. Domestic oil burning furnace (DOFA obtained from US EPA, RTP, NC) with a fixed concentration was used as a positive control and ultrapure water as a negative control.

2.4.3. ESR

Two different ESR measurements approaches were applied, one for the different filter extracts and an improved method for the quartz fibre filters without any extraction procedure (ESR-direct). Both approaches are based on the trapping of PM induced (hydroxyl radicals) OH• mainly generated via Fenton-type reaction in presence of H_2O_2 and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as spin trap. Transition metals are especially sensitive to H_2O_2 oxidation and generation of OH-radical.

The preparation and analyses of the extracted filter samples were done according to the method by Shi et al. (2003b) without the described filtering step of the sample after incubation and prior the ESR analyses. Briefly, PM suspensions are mixed with the chemical ingredients (H_2O_2 and DMPO), followed by incubation for 15 min at 37 °C in a heated shaking water bath prior to ESR analysis. The ESR quantification was conducted with the Analysis Software (2.0 or higher, Magnettech GmbH, Berlin) on first derivation of ESR signals of DMPO–OH quartet as the average of total amplitudes and expressed in arbitrary units (A.U.), expressed per sampled volume or per μg of PM. Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy) was used as an internal standard, and $CuSO_4$ as a positive control.

2.4.4. CRAT assay

The chemiluminescence reaction of acridinium ester under slightly basic conditions forms the basis of the CRAT assay. ROS production is measured from the interaction of reductants and oxidants (Zomer et al., 2011). The CRAT assay uses DTT as the reducing agent leading to formation of hydrogen peroxide, which in turn reacts with acridinium ester after addition of a buffer. The light emitted during this reaction is measured for about 1 s with luminescence meter. This assay is sensitive to oxidants such as ferric or cupric ions or organic species (e.g. quinones).

Briefly, 50 μL of sample with known concentration is incubated with 50 μL of 10 mM DTT for 10 min at 438 rpm in the plate reader. After incubation, 30 μL of acridinium ester (0.5 μg mL^{-1} in 0.1 M HNO_3) is added. The luminescence is measured in the kinetic mode after adding 50 μL 400 mM carbonate buffer pH 9.4 during 1 s. ROS production assay is performed using Mithras LB 940 Luminescence meter (Berthold) and 96 white micro plates. During the measurement the 1,2-Naphtoquinone (NQ) is used as calibration line. The results are quantified as equivalent of 1,2-Naphtoquinone per sample PM mass (pmol NQ μ^{-1}). 9,10-Phenanthrenequinone is used for quality control.

A more detailed methodology description of the assays is provided in the Supplement Information (SI).

2.5. Statistical analysis

Correlations for each OP method for different extraction methods and filter types were calculated using Spearman rank correlation coefficient (r_S). $p < 0.05$ was considered statistically significant. We did not differentiate between sampling site and sampling day due to too few data points. The paired sample *t*-test was used to examine whether filter type and extraction solvent differed significantly for each OP method.

3. Results and discussion

To investigate measurement precision, CVs for all duplicates used for OP analysis were calculated (See Table 1). Quartz filter extracts gave generally the highest CV values (15–38%), and the water extracted PM from Teflon filters the lowest (8–22%). The DTT and CRAT assay gave respectively the lowest and highest CV values. Corresponding CV values of the PM mass concentrations are shown in SI Table S 2.

3.1. Quartz filter versus Teflon filter

To our knowledge, no study has been published on the effect of filter type on oxidative potential of $PM_{2.5}$. Irrespective of assay type, OP m^{-3} values from quartz filters were significantly lower than those from Teflon filters (see Table 2). The AA and CRAT assay showed the largest difference in OP values, as the average OP^{CRAT} m^{-3} and OP^{AA} m^{-3} for quartz filters was 63% and 66% lower respectively. The DTT assay yielded the lowest decrease (21%) in OP values, while OP^{ESR} m^{-3} was 47% lower for quartz filters.

The attenuation of the OP values for the metal-driven assays (AA and ESR) might suggest lower extraction efficiency of the OP reactive components from the quartz filters compared to Teflon filters. OP values from the DTT assay were less affected, indicating that reactive components for this assay (e.g. organic species) were readily extracted from the quartz filters. The filtering of the quartz filters extracts, which was inevitable due to the high amount of quartz fibres, also led to removal of insoluble PM species. This might have contributed further to the attenuation of the OP values. Yet, as seen in Table 3 and Fig. 1, we found high correlations between OP values derived from Teflon and those from quartz filters. This suggests that the choice of sample collection media may have a profound effect on the absolute value for each OP assay, but not on the *relative* measure of OP reactivity for these four assays.

3.2. Methanol extracts versus water extracts

We only observed a significant effect of the choice of extraction solvent for the DTT assay ($p < 0.01$, paired t-test), with a lower OP^{DTT} m^{-3} for the water extracts. Water extracts were not analysed with the CRAT assay. As mentioned in Section 2.2, we did not perform water extraction on quartz filters, thus the comparison has only been done for Teflon extracts.

Several studies assessed the choice of extraction solvent on OP assessment. Eiguren-Fernandez et al. (2010) compared the DTT activity of ambient PM with two extraction methods for Teflon filters; dichloromethane extraction including filtering and water extraction. They found the water extracts to be over an order of magnitude more reactive than the dichloromethane extracts (Eiguren-Fernandez et al., 2010). Verma et al. (2012) measured the DTT consumption of water and methanol $PM_{2.5}$ extracts from quartz filters Both extracts were filtered, but the methanol extract still produced significantly higher DTT reactivity (expressed per μg of PM mass) than the water extract (Verma et al., 2012). Moreover, Rattanavarha et al. (2011) also tested out the difference in extraction solvent for the DTT assay, and found for 1,4-Naphtoquinone, a greater DTT consumption when extracted with methanol compared to water. Methanol and dichloromethane have similar extraction properties, but the former has higher polarity and is therefore also able to extract the hydrophilic compounds in addition to the hydrophobic organic components. Since we also did not filter the methanol extracts, the methanol-insoluble components are also retained. Daher et al. (2011) examined the DTT activity for different sampling methods and reported the highest OP^{DTT} mg^{-1} for the Biosampler, which is considered most efficient in collecting both the insoluble and soluble PM species. Consistent with our results, this suggests that the use of methanol to retrieve DTT reactive components might be more efficient than using water.

The choice of extraction solvent had no significant effect on the ESR and AA assay, showing that the OP reactive components (i.e. water-soluble transition metals) are equally effectively extracted with water as with methanol.

We also used ESR to assess OP for quartz filters without the extraction step (ESR-direct), which shortened analysis time. ESR-direct had the lowest OP values among the ESR assays. This might be caused by the fact that not all components are available for reacting with H_2O_2 (and subsequently DMPO). Despite the attenuation of the ESR-direct signal, the difference in OP between methanol quartz extract and ESR-direct was not significant. More importantly, ESR-direct was very highly correlated with OP^{ESR} m^{-3} and OP^{AA} m^{-3} measured from PM suspensions. This indicates that the ESR-direct may be a promising method to use in future OP studies.

3.3. Correlations between OP measurement methods

Furthermore, the effect of filter type and extraction solvent between the different OP methods by was explored by calculating Spearman rank correlation coefficient shown in Table 3. Generally, the correlations between different OP values were affected by filter type (lower for quartz) and less so by extraction fluid. As discussed in Section 3.1, the filtering of PM suspension of the quartz filters might be one of the reasons for the attenuated OP reactivity.

Two of the chosen OP assays, ESR and AA, are mainly sensitive towards the transition metals which trigger the formation of OH radicals (Godri et al., 2009; Shi et al., 2003b), and the strong correlations between OP^{ESR} and OP^{AA} confirms this. Similar strong correlations were found for the DTT and CRAT assay, which rely on organic compound-mediated, and the latter also to a certain extent metals (Zomer et al., 2011). To what extent metals influence the DTT reactivity is a complex issue yet to be solved (Charrier and Anastasio, 2012; Lin and Yu, 2011).

Few inter-assay comparisons have been published so far. A comparison study was done by Künzli et al. (2006) where OP was assessed for water extracted $PM_{2.5}$ Teflon samples from 20 European cities using the ESR with DMPO as spin trap and RTFL assay reporting the AA dependent OP value. A moderate correlation was found (Pearson's correlation, $r = 0.65$) between OP^{ESR} and OP^{AA}, consistent with our results, although we applied the simplified AA-only assay. We observed moderate ($r_S = 0.63$–0.69) to very high

Table 1
Coefficient of variation (CV) values for filter duplicates used in the different OP methods, expressed as OP m^{-3}. (Met = Methanol, Q = quartz, T = Teflon).

	Ascorbate (nmol AA s^{-1} m^{-3})			DTT (nmol DTT min^{-1} m^{-3})			ESR (A.U. m^{-3})				CRAT (pmol NQ m^{-3})	
	Met Q	Met T	Water T	Met Q	Met T	Water T	Met Q	Met T	Water T	Direct Q	Met Q	Met T
Duplicates	14[a]	15	15	14[a]	15	15	14[a]	15	15	14[c]	14[a]	15
CV (%)	22	21	17	15	11	9	19	29[b]	22	29	36	22

[a] Filter omitted from calculations due to technical problems during sampling.
[b] High CV caused by one poor duplicate with an individual CV of 82%; without this duplicate, the CV decreases to 13%.
[c] One ESR signal was below detection limit.

Table 2
Effect of extraction fluid and filter type on OP values for four assays. T = Teflon, Q = quartz, Met = Methanol, $n = 15$.

	Ascorbate (nmol AA s^{-1} m^{-3})			DTT (nmol DTT min^{-1} m^{-3})			ESR (A.U. m^{-3})				CRAT (pmol NQ m^{-3})	
	Met Q	Met T	Water T	Met Q	Met T	Water T	Met Q	Met T	Direct Q	Water T	Met Q	Met T
Mean	5.5[a]	16.1	18.4	2.3[a]	2.9	2.2[b]	2058[a]	3883	1777	3885	636[a]	1732
Std. dev	3.8	10.9	14.8	1.3	1.7	1.3	1204	2068	1026	2489	632	1419
Min	0.8	3.23	2.2	0.4	0.6	0.5	499	762	407	648	38	98
Max	12.3	35.0	43.5	5.3	7.2	5.2	4866	7961	3733	8176	2192	4488

[a] Filter type: tested against methanol-Teflon, paired t-test, significant at $p < 0.01$.
[b] Extraction solvent: tested against methanol-Teflon, paired t-test, significant at $p < 0.01$.

Table 3
Spearman's correlation coefficient between OP methods, the lower shaded area is expressed per m^3 and the upper part is expressed per μg ($n = 15$).

		Ascorbate			DTT			ESR				CRAT	
		Methanol Quartz	Methanol Teflon	Water Teflon	Methanol Quartz	Methanol Teflon	Water Teflon	Methanol Quartz	Methanol Teflon	Water Teflon	ESR Direct	Methanol Quartz	Methanol Teflon
Ascorbate	Methanol Quartz		0.94**	0.96**	0.04	0.53*	0.60*	0.92**	0.86**	0.88**	0.90**	0.10	0.36
	Methanol Teflon	0.94**		0.96**	0.03	0.61*	0.63*	0.93**	0.94**	0.93**	0.88**	0.22	0.51*
	Water Teflon	0.93**	0.95**		0.03	0.54*	0.62*	0.89**	0.87**	0.91**	0.91**	0.14	0.45
DTT	Methanol Quartz	0.30	0.21	0.27		0.36	0.48	0.20	-0.07	0.00	0.09	0.23	0.08
	Methanol Teflon	0.41	0.36	0.36	0.88**		0.72**	0.61*	0.50	0.49	0.40	0.61*	0.50
	Water Teflon	0.31	0.27	0.30	0.91**	0.97**		0.62*	0.58*	0.58*	0.55*	0.49	0.56*
ESR	Methanol Quartz	0.69**	0.63*	0.66**	0.79**	0.81**	0.80**		0.87**	0.93**	0.83**	0.31	0.54*
	Methanol Teflon	0.88**	0.91**	0.94**	0.38	0.49	0.44	0.76**		0.94**	0.87**	0.19	0.46
	Water Teflon	0.93**	0.95**	0.96**	0.34	0.46	0.41	0.75**	0.97**		0.81**	0.29	0.60*
	ESR Direct	0.94**	0.91**	0.94**	0.29	0.34	0.28	0.69**	0.90**	0.91**		-0.10	0.17
CRAT	Methanol Quartz	0.39	0.36	0.37	0.91**	0.93**	0.93**	0.85**	0.52*	0.48	0.35		0.70**
	Methanol Teflon	0.53*	0.56*	0.53*	0.63*	0.82**	0.81**	0.83**	0.64**	0.67**	0.51*	0.78**	

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

correlations ($r_S > 0.90$) between OP^{AA} m^{-3} and OP^{ESR} m^{-3} for both filter types and extraction solvent, with the highest correlation for Teflon filter extracts.

Mudway et al. (2011) compared the DTT, AA depletion from a complex RTFL assay, and the AA-only assay for methanol extracts from TEOM filters. They observed no significant correlations between DTT and the two AA assays as with our results, but found a quantitative association (Pearson's correlation, $r = 0.74$) between the simplified AA assay and the AA depletion from RTFL assay (Mudway et al., 2011).

We found no significant correlations between OP^{DTT} m^{-3} and OP^{AA} m^{-3}, or between OP^{DTT} m^{-3} and OP^{ESR} m^{-3}. This suggests that a combination of OP^{DTT} and OP^{ESR}, or OP^{DTT} and OP^{AA} assay, might provide complementary results regarding their oxidative properties.

3.4. Correlations between OP and PM chemical constituents

The results of the correlations between PM chemical composition and OP methods are shown in Table 4. Generally, we found no major impact of filter type and choice of extraction fluid on these correlations.

OP^{ESR} m^{-3} and OP^{AA} m^{-3} were strongly correlated with Cu, Fe, and EC, and moderately with Zn. These correlations were also found when OP is expressed per μg of PM for these two assays (see SI Table S 5). OP^{ESR} m^{-3} and OP^{AA} m^{-3} were also not correlated with PM mass concentration. High correlations between OP^{ESR}, OP^{AA} and the transition metal concentrations are consistent with previous observations (Boogaard et al., 2011; Godri et al., 2009; Künzli et al., 2006; Nawrot et al., 2009; Shi et al., 2003b).

OP^{DTT} m^{-3} correlated moderately ($r_S = 0.61–0.68$) with Cu, Fe, Mn and Zn, and highly ($r_S = 0.86–0.96$) with K, Br and OC. When expressed per μg of PM, no significant positive correlations were found for the latter, but the correlations with Cu and Fe remained. OP^{CRAT} m^{-3} correlated moderately to highly ($r_S = 0.66–0.88$) to the same elements as OP^{DTT} m^{-3}, with the exception of Mn. Both OP^{CRAT} m^{-3} and OP^{DTT} m^{-3} correlated very highly with PM mass concentration. This strong correlation with PM mass concentrations may also have contributed to the high correlations between DTT, CRAT and Br, K, and OC. This is obvious when the correlations are expressed per μg of PM (see SI Table S 5) and the mentioned correlations disappear. As seen in SI Table S 7, DTT normalised by PM mass resulted in low OP variation between the samples, which

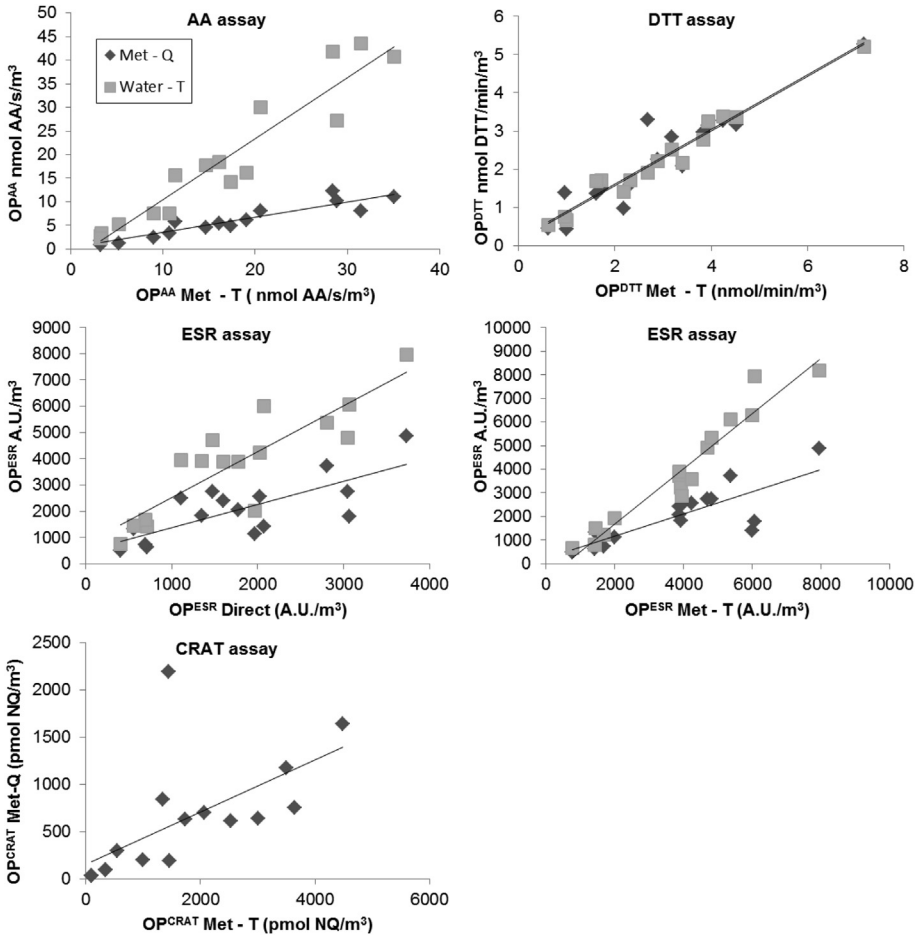

Fig. 1. Association between extraction methods and filter types for the different OP assays normalised by sampled volume. Correlation analysis from this figure is given in Table 3. Removing the outlier for CRAT assay results in an $r_S = 0.90$.

might have led to the high correlations to PM mass concentrations when expressed per m^3. Another reason of the high correlations might be the low sample variation for some of the PM components (see SI Table S 4).

The DTT assay has been reported to be predominately reactive towards PM quinone content and insensitive towards metals (Cho et al., 2005). However, recent studies have shown that DTT might be associated with the water-soluble metals, but the overall interactions

Table 4
Spearman's correlation coefficient between PM characteristics and OP methods ($n = 15$), expressed per m^3. (Met = Methanol, Q = Quartz, T = Teflon).

	Ascorbate			DTT			ESR				CRAT	
	(nmol AA s^{-1} m^{-3})			(nmol DTT min^{-1} m^{-3})			(A.U. m^{-3})				(pmol NQ m^{-3})	
	Met Q	Met T	Water T	Met Q	Met T	Water T	Met Q	Met T	Water T	Direct Q	Met Q	Met T
Mass Teflon	0.27	0.22	0.23	0.85**	0.95**	0.97**	0.72**	0.35	0.34	0.16	0.90**	0.78**
Mass quartz	0.29	0.25	0.26	0.85**	0.97**	0.98**	0.75**	0.37	0.38	0.20	0.91**	0.80**
EC	0.88**	0.86**	0.93**	0.38	0.35	0.32	0.70**	0.93**	0.90**	0.87**	0.41	0.46
OC	0.19	0.13	0.24	0.88**	0.95**	0.96**	0.80**	0.35	0.40	0.18	0.88**	0.76**
Al	0.43	0.25	0.39	0.80**	0.74**	0.72**	0.64**	0.35	0.35	0.39	0.69**	0.41
Br	0.29	0.27	0.33	0.77**	0.86**	0.88**	0.63*	0.41	0.39	0.19	0.79**	0.73**
Cr	0.30	0.25	0.29	0.57*	0.41	0.46	0.51	0.29	0.31	0.34	0.33	0.29
Cu	0.83**	0.80**	0.88**	0.62*	0.66**	0.63*	0.89**	0.91**	0.91**	0.83**	0.70**	0.73**
Fe	0.80**	0.79**	0.86**	0.63*	0.68**	0.64*	0.89**	0.89**	0.89**	0.81**	0.69**	0.74**
K	0.26	0.25	0.26	0.82**	0.87**	0.90**	0.71**	0.40	0.38	0.23	0.82**	0.79**
Mn	0.65**	0.51	0.59*	0.67**	0.65**	0.61*	0.69**	0.54*	0.54*	0.65**	0.55*	0.43
Ni	−0.34	−0.50	−0.38	0.15	0.13	0.20	−0.16	−0.41	−0.44	−0.40	0.01	−0.11
Pb	0.39	0.48	0.47	0.33	0.56*	0.54*	0.36	0.54*	0.51	0.25	0.48	0.52*
S	−0.15	−0.19	−0.21	0.72**	0.70**	0.78**	0.39	−0.13	−0.10	−0.23	0.70**	0.39
Si	0.34	0.21	0.31	0.56*	0.41	0.40	0.56*	0.36	0.37	0.41	0.50	0.35
Ti	−0.08	−0.18	−0.08	0.34	0.09	0.20	0.09	−0.13	−0.20	−0.12	0.05	−0.09
V	−0.21	−0.33	−0.25	−0.05	−0.10	−0.05	−0.29	−0.31	−0.37	−0.33	−0.15	−0.28
Zn	0.63*	0.63*	0.75**	0.63*	0.68**	0.67**	0.76**	0.79**	0.83**	0.66**	0.69**	0.66**

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

between DTT and metal ions have proven to be complex (Charrier and Anastasio, 2012; Lin and Yu, 2011; Ntziachristos et al., 2007).

The PM chemical constituents in our study are derived by ED-XRF, which only provides the total elemental concentrations in the samples. As mentioned earlier, due to pump failure, we did not have enough quartz filters to do elemental analysis for filter comparison. Less detailed information is provided with only ED-XRF, as the redox activities of PM associated elements depend on the chemical speciation and oxidative state of the metals (Shi et al., 2003a). However, the total elemental concentrations do offer an insight, albeit limited, on the interactions between the different OP assays and PM constituents, and also to which extent choice of extraction solvent and filter type affected these correlations. Furthermore, it was not within the scope of our paper to look at the complex interactions between the different OP assays and the various fractions of soluble/insoluble PM components.

4. Conclusions

We evaluated the effect of the choice of extraction solvent and filter type on four OP assays. Extraction solvent only had a significant effect on OP^{DTT}, but not on OP^{ESR} and OP^{AA}. We observed high correlations between OP^{DTT} and OP^{CRAT}, and between OP^{ESR} and OP^{AA}. These correlations were affected by filter type and to a lesser extent choice of extraction fluid. OP^{ESR} and OP^{AA} were highly correlated with Cu, Fe, Zn and EC, but not with PM mass. OP^{DTT} and OP^{CRAT} were highly correlated with PM mass, OC, Br, K, and S. These correlations were not affected by the choice of filter type and extraction fluid.

Despite the difference in extraction procedure, which likely led to the heavy attenuation of the OP values for the quartz filters, we still found strong correlations with OP m^{-3} values obtained from Teflon filter for each assay, and between the expected assay types. This indicates that the measurement of the relative OP reactivity is not greatly influenced by filter type for the four assays we applied in our study. These findings are promising for exploratory use in monitoring and subsequent epidemiological studies.

The ESR direct method, where ROS formation is measured directly on the filter, showed promising results, with high correlations to the ESR results from suspensions. By omitting the extraction step, analysis time is also shortened, which is an advantage for routine monitoring work.

Acknowledgements

We would like to thank John Boere, Paul Fokkens, Daan Leseman from RIVM, Kees Meliefste (IRAS) and Maciej Strak for helping with the sampling campaigns and lab work. We would also like to thank Saskia van der Zee (GGD Amsterdam) for assistance in locating the sampling site in Amsterdam. Lastly, thanks to Astrid John and Mareile Renker (IUTA) for performing the EC/OC analysis. The OPERA project is funded by the RIVM Strategic Research Program (SOR)

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atmosenv.2013.10.049.

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