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REVIEW- EVALUATING THE MOLECULAR ASSAYS FOR MEASURING THE OXIDATIVE POTENTIAL OF PARTICULATE MATTER

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Received. 28.2.2014.

Revised 24.6.2014.

Accepted 6.9.2014.

Part of this paper was presented at the Scientific Meeting Particulate Matter: Research and Management, WeBIOPATR Workshop & Conference, Belgrade, Serbia , October, 2013.

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Abstract

Several cell-free assays are currently used to quantify and detect the Reactive Oxygen Species (ROS). All of them have certain limitations, do not provide direct comparison of results and, to date, none of these assays have been acknowledged as the most suitable acellular assay and none has yet been adopted for investigation of potential PM toxicity. These assays include DTT, ascorbic acid, DCFH-DA and PFN assays which have been used in measurements of the particles generated from various combustion sources such as diesel engine, wood smoke (or biomass burning) and cigarette smoke, as well as for outdoor measurements. All the probes use different units for expressing redox properties of PM. Also, their reactivity is being triggered by different types of ROS. This limits the direct comparison of the results that are reporting the toxicity of the same aerosol type measured with various probes. This study is evaluating and comparing the various assays in order to develop deeper understanding of their capabilities, selectivity as well as improve understanding of the underlying chemical mechanisms.

Keywords: DTT, DCFH-DA, PFN, BPEA-nit, Ascorbic acid, oxidative potential

Highlights

- The available methodologies for the measurements of the OP of particles are reviewed.
- Atmospheric particles show higher OP than particles emitted from individual combustion sources.
- To have the most realistic values of OP several assays should be used simultaneously.
- A uniform way of reporting the results should be implemented.

Introduction

Reactive Oxygen Species (ROS) are molecules or ions which can be present on particulate matter (PM) (exogenous) or produced inside the cell as the by-product of the oxygen metabolism (endogenous). ROS include superoxide radical ($O_2\cdot$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$), alkoxyl radical ($RO\cdot$), carbon-cantered radical ($C\cdot$) and singlet oxygen ($\cdot O_2$). Increased production of ROS inside the cell affects existing balance between ROS and available antioxidants which further leads to numerous adverse health effects and oxidative stress. Biochemically ROS can damage cells by liquid peroxidation, oxidative modification of proteins and alternations in DNA.

To provide the rapid read out of the oxidative potential of PM, acellular assays are employed due to their low price and practicality, when compared to cellular assays. The only analytical method that provides direct quantification of radical species in the sample is electron paramagnetic resonance (EPR). In addition to the complexity and high price of this instrument, its low sensitivity is a major limitation. The use of Dithioteritol (DTT) to measure the ROS generation ability of particles has been first reported by Kumagai et al. (2002) [1]. Since then it has been extensively used for the measurement of PM oxidative capacity. Ascorbic acid is used mostly in cellular studies due to its antioxidant properties. However, as an acellular assay, it has been used in the limited number of studies to measure the oxidative potential of the particles generated from different sources.

DCFH-DA is a simple fluorescent compound which was initially developed in 1960s [2] for the measurement of ROS, specially H_2O_2. It has been used widely in both cellular and acellular studies.

Finally, profluorescent nitroxide compounds have been used successfully for the detection and quantification of the ROS generated from various combustion sources and atmospheric aerosols [3-7].

The tremendous amount of research conducted in this field calls for a comprehensive review that would facilitate the understanding of the limitations and challenges aerosol scientists are facing in this area. The main objective of this review is to present recent studies that examine the performances of various probes to measure the oxidative potential of PM, with special emphasis on their application as acellular assays.

Methodology of different assays

The Dithiotheritol (DTT) assay

The dithiothreitol (DTT) is a strong reducing agent which forms six membered ring with an internal disulfide bond when oxidised (Figure 1). The DTT quantitatively measures the formation of ROS by redox cycling chemicals such as quinones. Redox active species oxidize DTT to its disulfide form and donate an electron to the dissolved oxygen, forming superoxide. The DTT consumption is determined at several time points by measuring the remaining DTT with thiol reagent 5,5'-ditiobis-2-nitrobenzoic

acid (DTNB), which is followed by the production of 2- nitro-5-thiobenzoic (TNB) [1]. TNB is a coloured adduct produced in this reaction. It has a high molar extinction coefficient (14,150 $M^{-1}cm^{-1}$ at 412 nm) in the visible range and has to be measured within 2.0 h [8].

Figure 1

In order to report the DTT assay responses, two standardized units have been used: DTT activity (nmol DTT $min^{-1}\mu g^{-1}$) and normalized index of oxidant generation and toxicity (NIOG) [8]. DTT activity is expressed as the rate of DTT consumption per minute per microgram of sample. However, NIOG is expressed as the percentage of absorbance decrease ((Abs_0 - Abs´)/Abs_0*100)) per minute (T) and per microgram of particle sample (M), which is then normalized by the index of oxidation and toxicity (IOG) of 1,4 NQ ($IOG_{1,4\text{-}NQ}$) [10, 11].
Both DTNB and TNB are light sensitive [12, 13], so the reaction should be done in the dark hood. DTT is reactive towards limited number of species; it requires an additional step (reaction with DTNB) that may be a potential source of an experimental error and also the usage of this probe requires an incubation time of up to 90 min which may limit its application [9], especially if real-time ROS is desired.

Ascorbate acid assay

Ascorbic acid (AA) is a naturally occurring organic compound with antioxidant properties and is one form of vitamin C. This assay has been usually used *in vivo* and *in vitro* to determine the oxidative potential (OP) of the transition metals present in the PM. In this assay, Ascorbic Acid is using to reduce the metals ions and oxygen to finally generate hydroxyl radical. This high reactive radical reacts with a substrate such as salicylic acid to form the 2,3-and 2,5 dihydrobenzoates (DHBAs) [14] that can be monitored by using High Performance Liquid Chromatography (HPLC) technique. Figure 2 illustrates the reduction of particles by ascorbic acid to generate hydrogen peroxide.

Figure 2

In ascorbic acid (AA) assay, Particles are able to catalyse the O_2 consumption by ascorbic acid and can be monitored by the clark electrode [16]. Furthermore, OP of combustion-derived particles can be measured by photoluminescence (PCL) method in which the fast photochemical excitation of radical formations is combined with sensitive luminometric detection. In this method, determination of the OP is expressed as consumed ascorbate (nmol) per particle mass (micrograms) [17]. Moreover, the OP of extracted PM can be measured as the rate of depletion of ascorbic acid reacted with particles

and analysed by reversed phase HPLC [18] or by monitoring the absorbance of the AA at 265nm during the reaction time [19].

The Dichlorofluorescin Diacetate (DCFH-DA) assay

The use of 2',7'- dichlorofluorescin Diacetate (DCFH-DA) was first described by Keston et al. (1965)[20]. DCFH-DA is a fluorometric compound which has been synthesized and used for ROS measurements. It has been extensively used for visualising atmospheric ROS [21-23], cigarette smoke derived ROS [24, 25], diesel derived ROS [26], for determination of the intrinsic oxidative potential of nanoparticles [27] and also widely employed to measure the ROS activity of PM in the rat alveolar macrophage cells (*in vitro* studies) [28, 29].

The theory behind the DCFH-DA fluorescent increase is that nonfluorescent fluorescein derivatives will emit fluorescence after being oxidized by hydrogen peroxide [30]. The resulting fluorescence intensity is commonly converted into H_2O_2 equivalents against a calibration curve. DCFH-DA gets activated by sodium hydroxide to form DCFH that can be further oxidized to its fluorescent product 2', 7'-dichlorofluorescein (DCF) in the presence of reactive oxygen species (Figure 3). Upon excitation at a wavelength of 485±10 nm, yellow DCF emits intense fluorescence at the wavelength of 510±10 nm.

Figure 3

Hydrogen peroxide is capable of oxidizing DCFH just in the presence of a catalyst. Therefore, the horseradish peroxidase (HRP) enzyme is used to catalyse the generation of OH radicals and to improve the detection of target molecules (ROS). The presence of HRP in the reaction mixture induced a threefold increase in DCFH oxidation which can further lead to the over estimation of the measured OP [32].

It should be mentioned that DCFH-DA is prone to autooxidation. As a solution in ethanol and in dark, deacetylation of the DCFH-DA to DCFH is negligible [2], However, Photo-oxidation of DCFH has been observed in exposure to visible and UV light [33].

The Profluorescent Nitroxides assay

Since the late 1980s [34], the use of profluorescent nitroxide probes (PFN) for detecting and quantifying radical formation has expanded to include a wide range of applications (such as [35]). Several PFNs containing covalently linked fluorescence structure have been developed by Fairfull-Smith et al. (2008) [36]. These nitroxides are classified as profluorescent according to the fact that they normally emit weak fluorescence, but can be transformed into highly fluorescent form after a

simple chemical reaction. In other words, covalent linkage of a nitroxide moiety to a fluorophore efficiently quenches the excited states which lead to fluorescence emission [37]. After the reaction with ROS, this quenching is removed and the profluorescent nitroxide is transformed into its highly fluorescent form.

To detect the particle-derived ROS, 9,10-bis(phenylethynyl) anthracene (BPEA) was linked to a nitroxide-containing ring [38] to form BPEA-nit. BPEA-nit traps carbon-centered and sulphur-centered radicals, peroxyl and hydroxyl radicals (if the reaction is done in dimethyl sulfoxide (DMSO)) [39]. It has a fluorescence excitation at 430nm and emission at 485 and 513 nm which are long enough to avoid overlapping with any background fluorescence coming from compounds present in particles (e.g. polycyclic aromatic hydrocarbons (PAHs)).

This probe was applied to detect the ROS related to combustion- generated particles such as cigarette smoke [4], logwood stove and biomass combustion in a pellet boiler [3]; diesel exhaust [5, 6] and biodiesel exhaust [5]. Methane sulfonamide adduct was found to be the main fluorescent species produced from the reaction of the nitroxide with DMSO derived sulfoxy radicals. The unknown ROS concentration of particulate samples can be estimated by interpolating the fluorescence increase of nitroxide solution upon sampling from calibration curve made with different concentrations of methane sulfonamide adduct. Here, the usage of DMSO as a solvent is very important as the solvent is a mediator in reactions between various ROS and BPEA-nit. The mechanism is explained in more detail in Figure 4 [39].

Figure 4

Other assys

There are several other assays capable of detecting the free radical content of the particles. All of these probes display specific advantages and drawbacks, which made them less popular among aerosol scientists.

Electron paramagnetic resonance (EPR) can detect and quantify persistent radical species such as quinones directly, or superoxide and hydroxyl radical indirectly, by using a spin trap, due to their short life time [40]. Its ability to detect only the species with relatively long half-life makes it unsuitable for the real-time ROS monitoring. This method identifies the generated free radicals when specific spin traps or probes are used in the combination with specific reagents [41]. However, EPR is an expensive and complicated instrument which has low sensitivity due to low steady state concentration and short radical's lifetimes.

Dihydrorhodamine (DHR-6G) is the uncharged and nonfluorescent ROS indicator that can be oxidized to cationic, highly fluorescent rhodamine [42]. DHR-6G is reactive towards carbon-centered, peroxyl, alkoxy and hydroxyl radicals. Quantification is based on the concentration of the rhodamine

formed during the reaction of free radical with DHR-6G [43]. DHR-6G is air sensitive and photo sensitive compound, that yields significant background fluorescence.

Hydrogen peroxide and peroxides in general, can also be detected and quantified by para hydoxyphenyl acetic acid (POHPAA). This assay was introduced by Hasson et al. (2001) [44]. It is a fluorescent based assay which uses HRP to form the fluorescent POHPAA dimer for each hydrogen peroxide molecule present. The concentrations of hydrogen peroxide are determined by the comparison between the integrated fluorescence peak areas with those of standardized hydrogen peroxide solutions [45]. The reaction of POHPAA takes 5 minutes to complete [46] and the compound is not prone to auto oxidation upon exposure to light or air. However, POHPAA is reactive only towards strong oxidizing agents such as H_2O_2 which makes it impractical for the evaluation of the total OP of particles [47].

Table 1 illustrates an overview of the usage of the discussed assays. For each probe, presented are units, detection methodologies as well as PM sampling methodologies.

Table 1

All the probes use different units for expressing redox properties of PM. Also, as mentioned above, it can be observed that their reactivity is triggered by different types of ROS. This limits the direct comparison of the results that are reporting the toxicity of the same aerosol type measured with different probes. This is a very limiting factor that excludes the possibility of a complementary analysis. This way, researchers cannot make a database that would allow better understanding and a better insight into the processes behind the reported results.

Particle sampling approaches

To estimate the OP of PM and analyse chemical composition of PM, having a high efficiency sampling system is essential. Filter collection is a very common offline method which owes its popularity to its excellent collection efficiency, practicality and low-cost in spite of several drawbacks. In addition to the poor recovery of particles from the filters, extraction of the particles requires organic solvents which may produce further bias in the measurements. In addition, aging of the particles on filter surfaces can cause underestimation of ROS present. Furthermore, ultrasound agitation is commonly used to extract the particles from the filters. Ultrasound can cause chemical changes and thermal degradation of the chemicals including the chemical probes and particles [48].

To overcome the problems associated with filter sampling and minimise experimental errors, liquid impingement have been introduced. Liquid impingement enables particles to react directly and rapidly with their liquid quenchers. It is an appropriate choice when testing particle surface reactivity or when ageing can affect the chemical properties of the particles due to the long term sampling [49].

A particle into liquid sampler (PILS) is a potentially suitable online instrument for particle collection. Its principal is based on the growth of submicron particles in a condensational growth chamber that are consequently collected by a wetted cyclone. PILS is working with larger flow rates, compared to impingers, and can be used as a real- time ROS monitor. This methods allows the collection of very small particles and presents a promising methodology [50]. In another online sampling instrument presented by Fuller et al. (2014) [51], particles are collected on a hydrophobic filter and then washed with HRP solution at room temperature. The soluble components of the particles are extracted by DCFH-HRP assay in a continuous flow rate.

Furthermore, Versatile Aerosol Concentration System (VACES) has been developed by Kim et al. (2001) [52]. This instrument is capable of concentrating ambient particles of the coarse, fine, ultrafine size fractions simultaneously. VACES is usually connected to a liquid impinger to collect the particles in concentrated liquid suspension without substantial changes in their compactness or density. In the

sampling process, the concentration enrichment process decreases volatilization losses that are observed with the conventional particle collectors.

Significantly, different performance of various assays may also be due to the technique used for sampling. As stated earlier, it is of utter importance to use a proper sampling methodology that would allow particles to react directly and rapidly with the radical quencher, thus limiting possible changes of particles arising from the delay between sampling, extracting and analysis.

Discussion

OP has been adopted in an aerosol community as the measure of capacity of PM to oxidise target molecules inside the human body [53]. It is very important to establish a metric that represents the risk posed by particles, as it cannot be explained by just one parameter. OP is a suitable measure as it integrates numerous physico-chemical properties that all contribute to the overall toxicity of PM. In this regard, chemical composition, size and surface properties are mainly responsible for the resulting hazardness of PM. Surface area is specifically important as it presents an interface at which all of the biological and chemical processes take place.

Comparative studies

Presented cell-free approaches are used by researchers to explore OP of PM in a quantitative or semi-quantitative manner. As shown above, all of them have certain limitations, do not provide direct comparison of results and, to date, none of these assays have been acknowledged as the best acellular assay and none has yet been adopted for investigation of potential PM toxicity. Therefore, it is crucial to compare the performances of all the available probes that are used for evaluating the OP. This would provide information on the sensitivity, linearity and repeatability of each acellular probe and also, if the results from different probes can be comparable.

There are a limited number of studies aimed at getting information on the comparability of assays used for the measurement of the OP. A significant correlation of R^2=0.61 was observed in comparison of DCFH-DA (ROS macrophage) and acellular DTT assays by Hu et al. (2008) [54] while these two assays present quite low correlation in Cheung et al (2010) (Pearson's R =0.24) [55]. Another point that resulted from Hu et al. (2008) is that organic carbon (OC) is the most important effective component influencing the DTT activity of the particles. Janssen et al. (2014) [19] and Yang et al. (2014) [56] are both agreed that AA and EPR assays are highly correlated (R>0.9), while lower correlation was reported with DTT (R=0.4-0.6), specially for $PM_{2.5}$ particles. In the study of Sauvain et al. (2013) [57] on oxidative potential of metal oxide nanoparticles, while DCFH can be oxidized by almost all of the particles and AA is not sensitive enough, dissolution and complexation processes are able to additionally influence the measured reactivity as observed by DTT. DCFH specifically can

explain the O_2 reduction of the surface of the carbonaceous or Me/MeOx nanoparticles, while the chemical nature of DTT gives more insight into the biological oxidative potential of nanoparticles [57] and due to these independencies, a combination of DTT and DCFH-DA assays provides complementary information relative to the quantification of the OP [57, 58].

Sensitivity of different assays

The reason for the lack of the trends among various probes and approaches may be found in the study of sensitivity of these probes. DTT responded to the redox active organic compounds such as phenanthroquinone, while DCFH-DA was sensitive towards hydroperoxides, organic peroxides, alcohols, aldehydes and hypochlorite [47]. However, this study showed that although the response towards a specific functional group was observed, it was not linear. The concentrations used were also a bit higher than realistic ones.

Another review case study compared the performance of solid-phase supported profluorescent nitroxide scavenger, proxyl fluorescamine (PF) with the response given by DCFH [7]. Here, model aerosols used were aged nicotine and secondhand tobacco smoke. Reported results indicate that there was a large difference between the detected ROS levels. Authors argued that the reason for this may be the specificity of the PF probe which reacted with fewer free radical types (e.g. C·, N·, ROO·). The study has also introduced the solid phase trapped by PFN, as a new sampling method, which makes it comparable to the DCFH assay (ROS concentration: 3.2 and 1.4 $nmol.m^{-3}$ of equivalent H_2O_2 for PF and DCFH respectively).

Comparison of general trend

While it is not possible to directly compare the numerical values for OP obtained by different assays, there is a possibility of comparing the general trends reported for various aerosol types by a particular assay. Table 2 shows the reported OP of different combustion sources and atmospheric particles.

In general, the atmospheric particles have got higher OP than particles emitted from individual combustion sources. The loss of DTT when exposed to the urban atmospheric particles is obviously higher than in the case of diesel and wood burning (wildfire) PM. Sampling urban aerosols resulted in 0.09±0.003 nmol of DTT per µg of particles per minute [9], while diesel emissions displayed ROS activity in the range of 0.023-0.061 $nmol.\ \mu g^{-1}.\ min^{-1}$[59]. Furthermore, the OP measured by DTT is almost the same when two different approaches were used for sampling (filter and VACES) [59, 60].

The depletion of the ascorbic acid is greater in the reaction with wood burning particles than with diesel PM. Also, the OP value obtained from the reaction with atmospheric particles (4,680-15,120 $nmol\ AA.hr^{-1}\mu g^{-1}$) is remarkably high when compared to the other responses from AA which range

from 1.6-4.05 nmol AA.$hr^{-1}\mu g^{-1}$ for wood smoke and very low levels for diesel 0.3 nmol AA.$hr^{-1}\mu g^{-1}$. Collection of the particles on filters is the common method of sampling used in studies that employed AA.

BPEA-nit can quench particles generated from various types of sources. With one exception (stable cold start phase of the wood smoke which displayed a high OP of 4000±260 nmol/mg) , atmospheric particles, as measured with the BPEA-nit, still have the highest OP amongst all other sources of particles including cigarette smoke and combustion of diesel and biodiesel .

Due to lack of a uniform way of reporting results for DCFH-DA assay, the comparison of OP of different sources is difficult. The atmospheric OP has been reported as the concentration of the hydrogen peroxide equivalent per m^3 of air, while diesel PM OP is expressed as nmol of H_2O_2 equivalent per mass of the particles.

See et al. (2007) [61] compared OP of the fine particles ($PM_{2.5}$) generated from different combustion sources by using DCFH-DA. The study shows that on-road vehicle exhaust carries two times more ROS per mass than the outdoor air. Moreover, one stick of incense can emit significant amount of ROS around 0.17 nmol $H_2O_2/\mu g$, which is higher than cigarette smoke (sidestream) at around 0.13 nmol H_2O_2 /µg.

Table 2

The ROS content of atmospheric aerosols is surprisingly high in the case of all assays employed. The reason for this may be the aging of aerosols or the existence of persistent free radicals that are stabilized on the particulate surface as reported by Gehling et al. (2013) [68]. In this publication, authors argue that environmentally persistent free radicals (EPFR) can have lifetimes ranging from days to weeks and that can be formed during the reaction with metal oxides (such as CuO and FeO). The chemisorbed organic species like quinones, phenols or catehols have the ability of reducing these metal oxides bringing the metal into lower oxidation state. This can lead to the interaction between these reduced metals and EFPRs that can ultimately lead to the production of ROS while EFPR and the oxidised form of the transition metal are being regenerated.

Apart from the quenching ability of these probes, sampling technique employed can also alter the results reported. For example, although water soluble OC level of the filter and impactor are almost the same [69], depending on a particular aerosol type, the amount of semi-volatile organics and concentration of VOCs the errors originating from filter collection technique can vary. Another issue related to the interpretation of results would be the chosen extraction approach. If sonication is used to remove particles, positive artefacts may appear as ROS are normally generated during the sonication of the solvent [39].

Another contentious point that can be raised after analysing different assays and their applications is - *what would be the most appropriate metric to use in reporting OP results*?

Some researchers suggest that OP should be expressed as normalised per unit volume of air sampled OP/m^3 [19]. The argument is that this would be the most appropriate metric in terms of human exposure and epidemiological studies. However, such a metric would provide no information on the toxicity of a particular particle type or source of aerosol. As mentioned earlier, in addition to chemical composition, many other biologically relevant properties are related to the size, surface area and composition of PM. Smaller particles will normally give smaller mass but higher OP per unit mass (OP/mg of particles sampled) when compared to their larger counterparts. In addition, from toxicological point of view, smaller particles have larger surface area for the same mass concentration, stay longer in the atmosphere and can penetrate deeper into the lungs potentially making greater damage. Further when analysing the OP of different combustion sources, such as diesel vehicles, dilution is necessary prior to sampling. If the OP is normalised per volume of air instead of per mass of particles, it would depend on the dilution factor and therefore on the sampling conditions. While normalising the OP per volume of air would be most suitable for reporting ambient studies, when sampling close to or directly from combustion sources normalising per unit mass of particles sampled would be a more appropriate metric.

Conclusion

Current findings indicate that the detrimental effects of PM are the result of both their chemical composition and physical properties. It is still unclear which sources or constituents are in the direct causal relationship with observed effects. However, growing body of knowledge reports strong association between measured OP and organic component of PM. Black carbon is also playing an important role as a carrier of transition metals and semi-volatile components. Up to date, a number of different assays have been used, all with varying sensitivity, detection limits and technical requirements. To get a better understanding of the most realistic values of particulate OP and related contribution of different PM components and fractions, several assays should be used. A combination of the probes such as DTT/DCFH, DTT/BPEA-nit would provide complementary information on the ROS concentrations generated through a combination of different processes. Also, a uniform way of reporting the results should be implemented. Summary of recent studies reviewed here suggests reporting the atmospheric OP as (mol/m^3) and OP of particles measured in the vicinity or directly from combustion sources (tunnels, engines, etc.) as (mol/mg). A question yet to be answered is the contribution of different size fractions to the overall OP.

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Figure captions

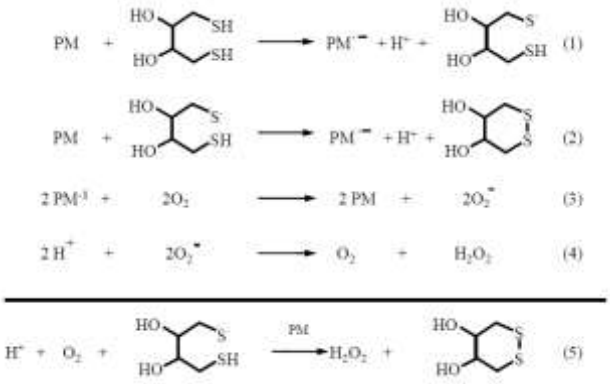

Figure 1: Chemical basis of the DTT (Dithiothreitol) assay [9]

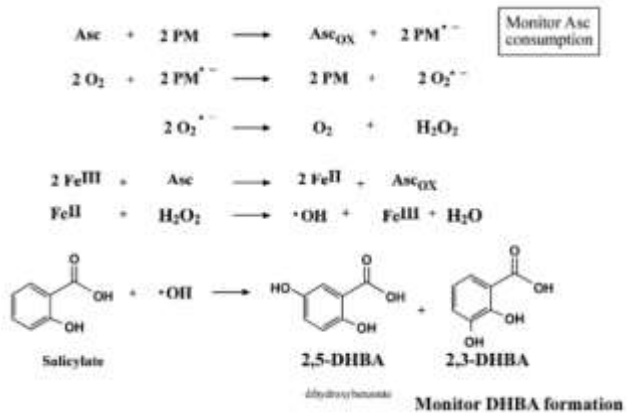

Figure 2: Chemical basis of the ascorbate acid reaction [15]

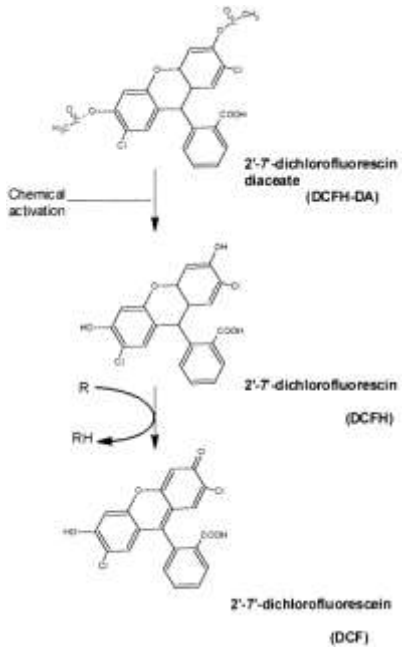

Figure 3: Proposed acellular mechanism of DCFA-DA [31]

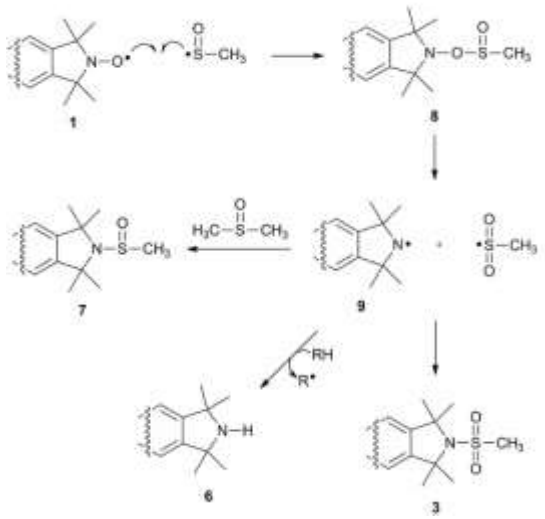

Figure 4: The reaction of BPEA-nit and ROS in presence of DMSO [39]

Table 1: Illustrates an overview of the usage of the discussed assays. For each probe, presented are units, detection methodologies as well as PM sampling methodologies.

	Unit	Sensitive to	Detection	Sampling methodology
DTT	nmol DTT.min^{-1}.μg^{-1}	-Organic species like PAHs and Quinones	UV absorbance	Filters VACES
Ascorbic acid	Rate of O_2 consumption ($nmol.min^{-1}.mg^{-1}$) nmol AA.s^{-1}.μg^{-1}	Transition metal	Clarke electrode [16] UV absorbance [19]	Filters
DCFH-DA	nmol H_2O_2 / m^3 nmol H_2O_2 / µg	ROS specially H_2O_2	Fluorescence Intensity	Impingement Filter PILS
BPEA-nit in DMSO	nmol BPEA-MSA/mg	C·, ·OH, ROO·, S·	Fluorescence Intensity	Impingement

Table 2: Evaluation of the oxidative potential of the various particle sources and their sampling methods

	Particle Sources				Atmospheric (Urban)	
	Cigarette	Wood smoke	Diesel			
DTT		0.024 nmol DTT.min^{-1}. µg^{-1} [58]- wild fire *(Filter)*	0.023-0.061 nmol DTT.min^{-1}. µg^{-1}[59] *(Filter)*	~ 0.020 nmol DTT.min^{-1} .µg^{-1}[60] *(VACES)*	0.06 (urban background)-0.13(traffic) nmol DTT.min^{-1}. µg^{-1} [19] *(filter)*	0.09±0.003 nmol DTT.min^{-1}. µg^{-1}[9] *(VACES)*
			0.039±0.05 nmol DTT.min^{-1}. µg^{-1}[62] *(Filter)*			
DCFH-DA	Main stream: 108±2 nmol H_2O_2/cigarette [24] Sidestream: 61.6± 7.9 nmol H_2O_2/cigarette *(Impingements for gas phase and filters for particle phase)*	13.5±1.30 nmol H_2O_2 /mg-incense smoke [63]*(Filter)*	0.06-0.26 nmol H_2O_2/µg [26]*(Filter)*		8.3±2.19 nmol H_2O_2/m^3 [64]*(PILS)* 0.02-3.81 nmol H_2O_2/m^3 [65] *(Filter)*	5.9±1.70 nmol H_2O_2/m^3 [23] *(MOUDI^{TM} -Filter)*
	~0.13 nmol H_2O_2/ µg (*Filter)*[61]	~0.17 nmol H_2O_2/ µg – incense smoke *(Filter)* [61]			~0.26 (outdoor)-0.45(traffic) nmol H_2O_2/ µg *(Filter)* [61]	
Ascorbic Acid		83.37±14.2% loss of AA in 50µg/ml [18] (1.6- 4.05 nmol AA.hr^{-1}. µg^{-1}) *(Filter)*	< 60.6±2.5% loss of AA in 100 µg/ml [66] (0.3 nmol AA.hr^{-1}. µg^{-1}) *(Filter)*		1.3 (urban background)-4.2 (traffic) nmol AA.s^{-1}. µg^{-1} [19] (4,680-15,120 nmol AA.hr^{-1}. µg^{-1}) *(Filters)*	
BPEA-nit	Mainstream: 101.4±29 nmol BPEA-MSA/cigarette [4] Sidestream: 15-52 nmol BPEA-MSA/cigarette *(Liquid Impingers)*	170- 4260 nmol BPEA-MSA/mg [3] *(Liquid Impingers)*	50-420 nmol BPEA-MSA/mg [5] *(Liquid Impingers)*		75-1183 nmol BPEA-MSA/mg [67] *(Liquid Impingers)*	