Review

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Abstract: Recent years have seen a significant increase in the scientific literature related to various methods for analyzing oxidative potential (OP) of atmospheric particulate matter (PM). The presence of several types of PM, differing chemical and physical properties, released by both anthropogenic and natural sources, leads to numerous health issues in living organisms and represents an attractive target for air quality monitoring. Therefore, several studies have focused on developing rapid and self-operative tests, employing different target molecules to assess OP of atmospheric aerosols as well as unique approaches to overcome some of the most common laboratory-related issues in this kind of analysis. This work provides an overview of online and automated systems, as well as a broad picture of the state-of-art of the various devices and methods developed on this topic over the last two decades. Moreover, representative studies on this subject will be discussed, analyzing the advantages and drawbacks of the developed automated techniques.

Keywords: particulate matter; oxidative potential; on-line; automated; dithiothreitol; 2′,7′-dichlorofluorescein; spectrophotometry; air quality; monitoring

1. Introduction

1.1. Particulate Matter and Induced Oxidative Stress

Air pollution affects the health of people, especially in urban areas, causing negative respiratory and cardiac effects, as shown by epidemiological and clinical studies [1]. Among air pollutant components, particulate matter (PM) has a pivotal role in inflammatory progression and induced toxicity, and continuous exposure is correlated to an increase in mortality [2,3]. During the last few decades, epidemiological and clinical research has demonstrated strong links between atmospheric aerosols and adverse health effects, including cardiovascular and respiratory morbidity [4], impaired pulmonary function [5], neurodegenerative disorders [6], and premature mortality [7]. PM is a mixture of small particles and liquid droplets, released into the atmosphere by natural process such as forest fires, Saharan dust, or anthropogenic activities as industrial processes, biomass burning, poor household waste management, as well as traffic-related sources and abrasion of brakes and tires [8–10]. Airborne PM released by industries and car exhaust is responsible for the generation of biological oxidative stress [11,12], which is an imbalance in the cellular redox homeostasis due to excessive reactive species production or weakening of the antioxidant defence system. Oxidative stress is thought to be an important underlying mechanism of action for the outcome of PM induces adverse health effects [11]. The mechanisms by which PM induces oxidative stress include the generation of Reactive Oxygen Species (ROS) and the inhibition of the intracellular enzymatic antioxidant defences [13]. When ROS levels exceed the cellular antioxidant capacity, a cascade of events is triggered in the cells, resulting in inflammation and significant cellular damage [14–16]. It has been shown

that PM consists of different chemical constituents including H_2O_2, organic peroxides, and nitrite peroxides, ions such as hypochlorite ion (OCl^-), peroxide anion (O_2^-), radicals such as hydroxyl ($^\bullet OH$), superoxide radicals ($^\bullet O_2^-$), and organic peroxyl ($ROO^\bullet$), including aromatic compounds such as polycyclic aromatic hydrocarbon (PAH) and transition metals, which can produce oxidative damage to lipids, proteins, and DNA [5,17–20].

1.2. OP of PM as Target for Rapid Environmental Evaluation

For the above-mentioned reasons, PM is a remarkable target for air quality analysis. Therefore, to rapidly assess the presence of particles with adverse health effects in environmental samples, one of the promising procedures is the evaluation of OP oxidative potential. This characteristic is more closely related to negative health effects and biological responses [21], thus it could be more informative than other metrics such as mass concentration [22,23]. The quantitation of this parameter has many potential advantages compared to cellular assays, such as low maintenance, no risk for microbial contamination, and the portability of the system. OP measurement evaluation has been introduced by Cho et al. 2005 [24], which proposed Dithiothreitol (DTT) reactivity as a quantitative acellular probe for the assessment of the capacity of the atmospheric PM to catalyse ROS generation. DTT is a strong reducing agent that forms a six-membered ring with an internal disulphide bond when oxidized. The DTT assay is mainly sensitive to organic compounds, hulis, and quinones [25,26]. Successively, several other chemical assays have been used to measure OP, employing glutathione, cysteine, or the ascorbic acid (AA) assay, mainly sensitive to transition metals [27–29]; the DCFH (dichlorofluorescein), or additionally, the electron paramagnetic resonance methods (EPR) [26]. Traditional techniques of quantifying aerosol OP are time-consuming and labour-intensive, with large delays between aerosol sampling and ROS assessment. The offline determination of OP with the mentioned assays is relatively time-consuming, which represents a significant limitation for fast environmental risk evaluation and management. Moreover, the typical sampling procedure employs manual methods, where sample chemical analysis is usually conducted after the aerosol is collected on filters or other supporting devices. However, these approaches delay the analysis of short-lived redox active species related to the aerosol, including H_2O_2, organic peroxides, and radicals, which might be biochemically more active when collected than the elements measured after days or weeks of storage [30,31]. This loss might lead to substantial undervaluation of the ambient particle-bound ROS concentrations. To overcome this problem, the analysis can be completed in the field immediately after collection, however, this would make the processes extremely labour intensive, challenging to implement, and unfeasible for an extended sampling period (e.g., 24 h) which is representative of most sampling programs. A key challenge for researchers and monitoring programs is the difficulty of obtaining fast results to complete long-time series. Therefore, a growing number of automated devices based on acellular assays have been researched and developed to enable quick and high-throughput OP analysis due to the lack of a reliable and robust way to measure OP and ROS in real-time and on a regular basis [26,30,32–35].

1.3. Literature Overview of Online Methods

As can be seen in Figure 1, the number of works on the analysis of OP via automated and online approaches has been increasing during the last 15 years. The results of Figure 1 were obtained using the Scopus search tool, combining different entry objects, and using different keywords (such as online and on-line). In addition, some further pertinent articles were found in the cited references.

Literature offers several articles worth mentioning on online instruments which have made relevant progress in the field, as well as opened new perspectives for further improvements other than facilitating real-time and automated characterization of particles released in the atmosphere and their oxidizing properties, along with their potential origin [36–42]. The increase in the number of publications in this field suggests that this review work could be timely and can be useful to scientists providing a summary of the progress and

challenges of OP analysis based on automated or online measurements. This review provided an overview of the automated and online systems for the assessment of the oxidative potential of atmospheric particulate matter. The most noteworthy automatic and/or online detection systems mainly based on DCFH and DTT assays were discussed, as well as the advancements achieved over prior studies and with newly employed redox molecules. A summary of the performances of the examined systems, including the relative limit of detections, linear ranges, and flows used during PM collection or analysis were discussed highlighting the importance of rapid target evaluation.
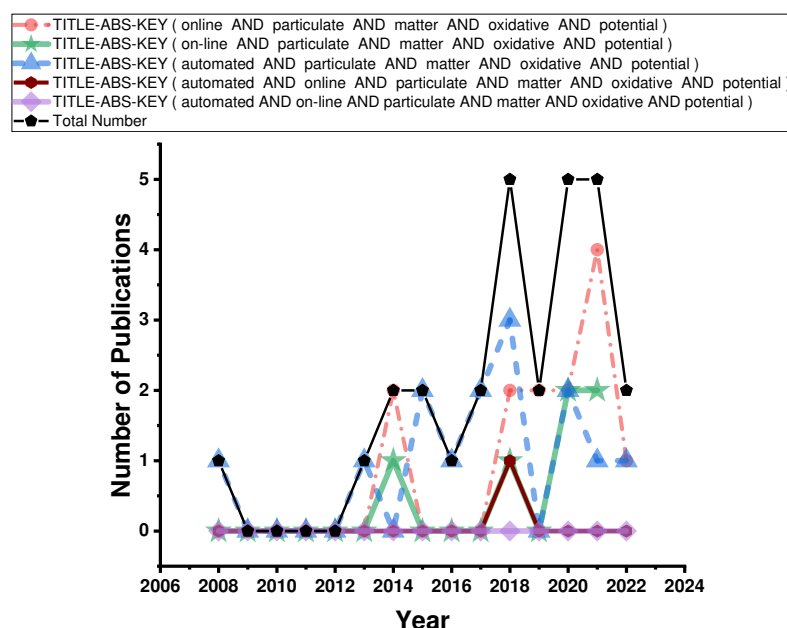

Figure 1. Graphic result of Scopus literature research, using different keyword combinations. The total number of articles (black curve) has been calculated removing duplicated articles.

2. Automated and OnLine Analysis Systems Based on DCFH

2.1. DCFH Method to Assess OP

A widely used method for ROS detection is based on the oxidation of the non-fluorescent probe $2',7'$-dichlorodihydrofluorescein ($DCFH_2$), to generate the highly fluorescent $2',7'$-dichlorofluorescein (DCF). Although this test is usually applied for ROS detection in cellular methods [43], in 1983 Cathcart et al. [44] also used it for the analysis of micro and pico-quantities of hydrogen peroxide, and thereafter Antonini et al. [45] and Hung et al. [46] employed this assay for acellular quantification of reactive oxygen species from particles. Result of this assay can be considered a general indicator of the particle-bound ROS, because the oxidation of $DCFH_2$ is non-specific and it is in competition with endogenous antioxidants from biological systems. Although this is still a viable and commonly used approach, it was established more than 50 years ago [47], hence it lacks speed and portability when considering the current demand for fast OP analysis. However, automated methodologies have been developed to perform this assay (Table 1). One of the first automated systems for continuous sampling of ambient aerosol and ROS concentrations assessment was developed in 2008 by Venkatachari and Hopke [30]. They developed an integrated sampling-analysis system based on collecting atmospheric particles in an aqueous slurry and detecting ROS concentration using the DCFH fluorescence method. The results of the lab-scale investigation of the ROS sampling-analysis system revealed that the prototype continuous system could detect particle-bound ROS in a range between 81 and 668 nM with a sample residence time in the flow reactor between 18.5 and 33 s respectively while accounting for short-term variations. Although this system showed good sensitivity, portability remains a non-negligible drawback.

Table 1. Acellular Online Automated methods for OP PM measurement.

Quantitation Method	On-Field Application	Validation on Real Samples	Automated	Online	Dynamic Range	Linear Range	Collector Flow	Flow during Analysis	LoD	Link
fluorescence	No	N.A.	Yes	Yes	50 to 800 nM	0.051 to 0.8 μM	14–25 L/min	N.A.	81.1 nM	[30]
fluorescence	Yes	Yes	Yes	Yes	N.A.	0.1–0.4 μM H_2O_2	16.7 L/min	N.A.	N.A.	[32]
fluorescence	Yes	Yes	Yes	Yes	N.A.	0.01–0.2 μM	20 L/min	0.4 mL/min	0.28 nM H_2O_2 equivalents	[48]
fluorescence	Yes	Yes	Yes	Yes	4–2000 nmol $[H_2O_2]$ equivalents/m^3 air	0.025–1.0 μM (calibration with H_2O_2)	5 L/min	N.A.	3.85 nmol $[H_2O_2]$ equivalents/m^3 air	[39]
fluorescence	Yes	Yes	Yes	Yes	N.A.	0.1–0.4 μM (calibration with H_2O_2)	16.7 L/min	N.A.	gas-phase ROS: 0.16 nmol H_2O_2/m^3; particle-phase ROS: 0.12 nmol H_2O_2/m^3	[38]
fluorescence	Yes	Yes	Partially	Yes	N.A.	0–0.15 μM	1.7 L/min	0.3 mL/min	1.3 nmol/L (offline); 2 nmol/m^3 (online)	[41]

N.A.: not applicable.

2.2. On-Field Methods for PM Analysis

On the base of the system mentioned in the previous section, some laboratory and field tests were performed by Hopke et al. [30] to develop an effective automated particle-bound ROS sampling-analysis system. Their approach employs the DCFH fluorescence method: $PM_{2.5}$ was collected into an aqueous slurry containing the DCFH solution, by using a sharp cut cyclone and a particle-into-liquid-sampler (PILS). Laboratory results showed that the DCFH and H_2O_2 standard solutions could be kept at room temperature for at least three days. The average ROS concentration measured at urban sites in the USA was 8.3 ± 2.2 nmol m^{-3}, which is within the range of commonly reported values. This study was the first automated sampling-analysis system to measure ambient particle components on field. DCFH was also used by King and Weber [48] for an online, semi-continuous instrument to measure fine particle ($PM_{2.5}$) ROS. To obtain total ROS (ROSt), which consists of gas plus particles, the online technique included a mist chamber scrubber, alternating with gas phase ROS (ROSg) by means of an inline filter. Particle phase ROS (ROSp) was determined by the difference between ROSt and ROSg. During various seasons, the sensor was deployed in both urban Atlanta, Georgia, and a rural site. The analytical Limit Of Detection for this system was 0.28 nM. In 2016 Wragg et al. [39] introduced a portable device for measuring particle-bound ROS in real-time, using DCFH and horseradish peroxidase (HRP), via fluorescence spectroscopy. All components of the new instrument were attached to a containing shell, resulting in a compact system capable of automated continuous field deployment over many hours or days. According to laboratory experiments, the instrument has a detection limit of 4 nmol H_2O_2 equivalents per m^3 air, a dynamic range of at least 2000 nmol H_2O_2 equivalents per m^3 air, and a temporal resolution of 12 min. Additionally, it can measure for up to 16 h if left unattended. The Gas and Aerosol Collector (GAC-ROS) system, for simultaneous measurement of reactive oxygen species (ROS) in both gas and particle phases, was developed by Huang et al. [38]. This method is based on DCFH assay to provide fast sampling and analysis of atmospheric ROS. The GAC-ROS, which consists of a Gas and Aerosol Collector (GAC), a series of reactions, a transportation system, and a fluorescence detector, was tested for instrumental performance in the laboratory. The comparison of online and offline methods for particle-bound ROS showed a significant loss of ROS due to the relatively long-time offline treatment period. The calibration curve results were successful, with an R^2 value above 0.998, and low LOD: gas-phase ROS: 0.16 nmol H_2O_2 m^3; particle-phase ROS: 0.12 nmol H_2O_2 m^3. This study was the first to simultaneously obtain concentrations of gas and particle-phase ROS using an online method. An online and offline ROS analyser employing dichlorofluorescein (DCFH) assay was designed by Zhou et al. [41]. The system was calibrated with H_2O_2, and its sensitivity was characterized using potential interference compounds (O_3, NO_2, SO_4^{2-}, NO_3^-, Fe^{3+}, Fe^{2+}). In comparison to previous studies, the authors improved the water and PBS degassing to prepare working solutions; the separation of DCFH and peroxidase working solutions, and they were able to avoid ultrasonic filter extraction for offline analysis. The detection limit for the offline and online analysis was determined as 1.3 nmol/L and 2 nmol/m^3, respectively. The ROS concentration of several aerosol types, including ambient aerosols as well as fresh and aged aerosols from wood combustion emissions, was determined using both online and offline techniques. The stability of the ROS was assessed by comparing the ROS concentration measured by the same instrumentation online in situ with offline measurements. Interestingly, the ROS evolution in specific samples was evaluated by conducting the analysis after storage times of up to 4 months. ROS stability was found to decrease as storage time increased. This study demonstrated that on average, 60% of the ROS were highly reactive and vanished over the filter storage period, emphasizing the importance of quick online ROS assessment.

3. The Instrument Particle into Nitroxide Quencher PINQ

This section discusses the performance of an instrument for the rapid quantification of PM-bound ROS that employs a different molecule as a probe, developed by

Brown et al., 2019 [42]. The Particle Into Nitroxide Quencher (PINQ) is a steam collection device where the sample aerosol is continuously mixed with a stream of water vapour generating a supersaturated mixture. Particles are then formed into large liquid droplets to achieve high-efficiency capture independently of initial particle size. The grown droplets are continuously collected into a liquid vortex within a vortex collector (Figure 2). This was designed over a simpler impaction system as it allows the capture of insoluble in the collection liquid. The vortex collector is solvent resistant and is used to visually confirm the liquid vortex. PINQ used the 9,10-bis (phenylethynyl) anthracene-nitroxide (BPEAnit) ROS assay in conjunction with an insoluble aerosol collector (IAC). With a collection efficiency of >0.97 and a cut-off size of 20 nm, the IAC constantly gathers PM of any size or chemical straight into a liquid sample. The sampling time resolution of the PINQ is 1 min, with a LOD of 0.08 nmol/m^3 in equivalent BPEAnit-Me concentration per volume of air. The combination of the highly concentrated IAC liquid sample, the reduced liquid sample volume, and the quick response and stability of the BPEAnit probe enabled high sample time resolution and sensitivity.
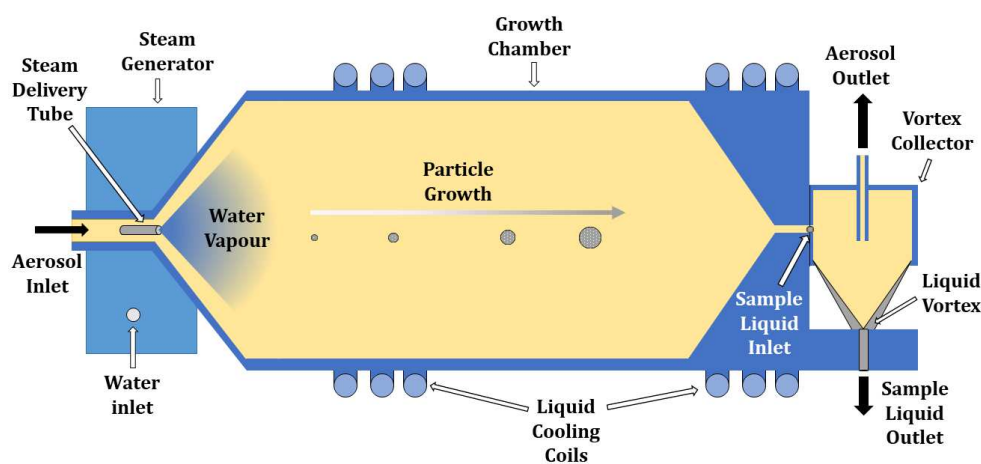

Figure 2. PINQ insoluble aerosol collector (IAC) diagram, highlighting major components such as the aerosol input, steam generator, growth chamber, and vortex collector. (Figure adapted from Brown et al. [42]).

4. Automated and OnLine Analysis System Based on Ascorbic Acid

Ascorbic acid (AA), an antioxidant prevalent in the lung, is a remarkable redox molecule used to perform cellular [49] and acellular [50,51] assays for OP measurement of PM. In this method, the redox-active species present in PM oxidize the reagents and the oxidative potential is quantified as the rate of AA depleted, measured with spectrophotometric techniques. This chapter explores the efficiency and functionality of an online system proposed by Campbell et al. [52]. In their work, the authors adapted the method developed by Wragg et al. [39], combining the benefits of online continuous monitoring with a physiologically relevant test. In this instrument, particles are drawn into the aerosol collector at 5 L/min, through a charcoal denuder to remove volatile organic compounds (VOCs). Particles are collected onto a wetted filter and extracted into the AA solution. The reaction mixture is then injected through a reaction coil for 20 min, with the reaction bath maintained at 37 °C (Figure 3). Oxidizing species present in the secondary organic aerosol (SOA) may react with the ascorbic acid to produce dehydroascorbic acid (DHA), the resulting product is then mixed with o-phenylenediamine (OPDA) in 0.1 M HCl. In this acidic environment, the DHA reacts rapidly with OPDA to give the fluorescent product 3-(1,2-dihydroxyethyl)fluoro[3,4-b]quinoxaline-1-one (DFQ), which can be used to estimate the degree of AA oxidation and hence the OP of the aerosol sample. The concentration of DFQ, related to aerosol OP, is determined by fluorescent spectroscopy.
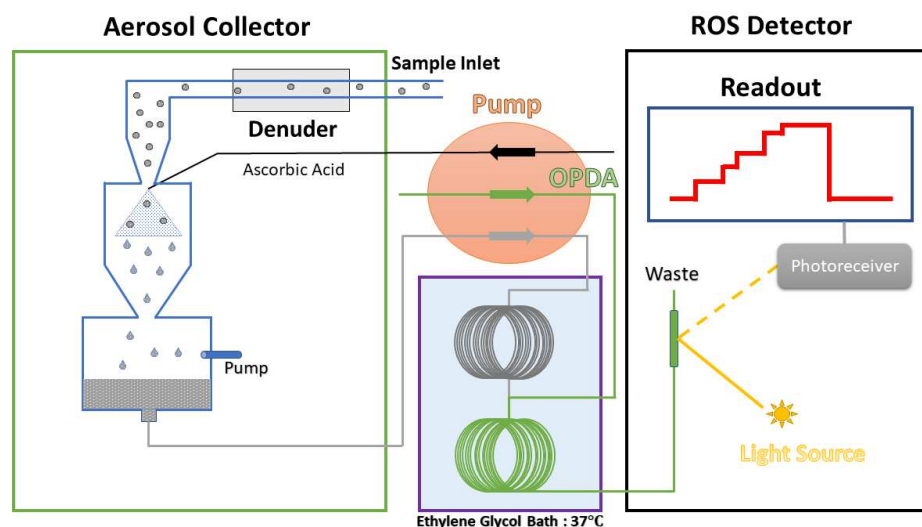
Figure 3. Simplified schematic illustrating the online AA method. (Adapted from Campbell et al. [52]).

The method LODs were estimated for a range of atmospherically significant chemical components as follows: Cu(II) 0.22 ± 0.03 µg/m^3, Fe(II) 47.8 ± 5.5 µg/m^3, Fe(III) 0.63 ± 0.05 µg/m^3, and secondary organic aerosol 41.2 ± 6.9 µg/m^3. These are relatively high values for typical atmospheric conditions; however, authors suggested that the online AA approach might be useful for studying particle-bound ROS in polluted urban environments as well as in smog chamber investigations.

5. Automated and Online Analysis Systems Based on DTT Assay

As mentioned above, one of the most widely reported methods for assessing PM oxidative load is the dithiothreitol (DTT) assay. DTT has been employed as a surrogate of biological sulphurs that can be oxidised when exposed to ROS due to its two thiol groups. The presence of transition metals and quinones in PM can often be linked to DTT consumption as they can catalyse the oxidation of DTT via catalytic redox processes. In the DTT assay, the OP is evaluated as the consumption rate of DTT over time due to the redox active species in PM extracts. This parameter, defined as DTT activity, is commonly reported as nmol of DTT consumed in time (nmol/min) or as OP referred to the volume of sampled air (nmol/min·m^3). Many innovative, robust, and user-friendly technologies for performing this assay have been developed during the last 20 years. This section explores and discusses some of the cutting-edge automatic and/or online systems employing DTT for OP analysis, by reviewing the progress over time (Table 2).

One of the first online systems was developed in 2014 by Koehler et al. [53] and presented a DTT assay that couples a particle-into-liquid-sampler with microfluidic-electrochemical detection. This method allows high temporal resolution monitoring of PM reactivity, decreasing the traditional detection limits. To compare traditional and online DTT techniques, an urban dust sample was aerosolized in a laboratory test chamber at three atmospherically relevant concentrations. The online system gave a stronger correlation between DTT consumption rate and PM mass ($R^2 = 0.69$) than the traditional method ($R^2 = 0.40$) and increased precision at the high temporal resolution, reaching a limit of detection of 2 pmol/min.

Table 2. Automated and online systems based on DTT assay.

Quantitation Method	On-Field Application	Validation on Real Samples	Automated	Online	Linear Range	Collector Flow	Flow during Analysis	LoD	Link
Electrochemical	No	Yes	Yes	Yes	10–100 µM	12.5 L/min	N.A.	2.49 ± 0.20 µM	[36]
Electrochemical	No	Yes	semi-automated	Yes	N.A.	13 L/min	0.18 mL/min	2 pmol DTT/min (aerosol concentration of 10 µg/m^3)	[53]
Absorbance	Yes	Yes	semi-automated	No	0.3–1.9 nmol/min	1.13 m^3/min	N.A.	0.31 nmol DTT/min	[33]
Absorbance	Yes	Yes	Yes	No	0.22–1.25 nmol/min (from graph)	1.13 m^3/min	N.A.	Water-soluble metals method = K: 0.03 mg/L; Mn: 0.00007 mg/L; Fe: 0.009 mg/L; Cu: 0.0002 mg/L Total metal method = K: 0.03 mg/L; Mn: 0.0002 mg/L; Fe: 0.02 mg/L; Cu: 0.002 mg/L	[34]
Colorimetric	Yes	Yes	No	No	0–12.5 nmol DTT	4 L/min	N.A.	7.92 ± 0.3 ng NQ	[54]
Absorbance	Yes	Yes	Yes	Yes	0.025–0.25 µM	3.0 L/min	continuous-flow DAD	0.15 nmol/min	[37]
Absorbance	Yes	Yes	Yes	Yes	0.03–0.23 µM/min	42 L/min	N.A.	0.24 nmol/min	[40]
Absorbance— Electrochemical	No	Yes	semi-automated	No	0–100 µM DTT	16.7 L/min	0.085 mL/min	UV/vis: 0.150 mAU · min/µM DTT Electrochemical: 0.218 Ip/µM DTT	[35]
Electrochemical	No	Yes	No	Yes	Amperometric (BIA): 0.75–200 µM Amperometric (FIA): 10–500 µM	N.A.	1.5 mL/min	Amperometric (BIA): 0.75 µM Amperometric (FIA): 1.5 µM	[55]

N.A.: not applicable.

A semi-automated system for quantifying the oxidative potential of aerosol liquid extracts using the DTT assay was conceived by Fang et al. [33]. This device analyses one sample per hour autonomously and has high analytical accuracy (coefficient of variation of 15% for positive control, 4% for ambient samples) and a low limit of detection (0.31 nmol/min). On ambient samples, the automated technique and the manual method produced a good correlation (slope = 1.08 ± 0.12, R^2 = 0.92, N = 9). The system was used to collect substantial data on DTT activity for the Southeastern Center for Air Pollution and Epidemiology (SCAPE). The ambient particles had been collected from different environments (urban, roadside, and rural) in the southeastern US. The water-soluble $PM_{2.5}$ DTT activity on a per-air-volume basis was spatially uniform and often well correlated with $PM_{2.5}$ mass (r = 0.49 to 0.88). Although large-scale toxicological and health studies involving aerosol exposure is needed, this result indicated a plausible mechanistic explanation (oxidative stress) for reported $PM_{2.5}$ mass-health relationships. The variability in the DTT activity associated with aerosols from sources that vary with season was highlighted by the heterogeneity in the intrinsic DTT activity (per-PM-mass basis) across seasons.

Inspired by the work of Fang et al. [33], Gao et al. [34], developed an automated analytical system for measuring the oxidative potential with the DTT assay of filter extracts, including both water-soluble and water-insoluble (solid) aerosol species. This study highlighted that both soluble and insoluble aerosol components contributing to OP are largely secondary, while the results were significant with roadway emissions as sources of DTT-quantified $PM_{2.5}$ OP. They compared three approaches for measuring total oxidative potential. Methanol was used as solvent with (1) and without (2) filtering the extract, and (3) extracting the sample in pure water, performing the OP analysis in the extraction vial with the filter. The water extraction approach gave the highest DTT responses with the greatest accuracy (coefficient of variation of 1–5%) and was associated with a higher number of other aerosol components. As a result, the third approach was used in the field investigation to determine the total OP. For two 1-month research periods, daily 23-h filter samples were collected at a roadside (RS) and a representative urban (Georgia Tech, GT) location, and both water-soluble (OP^{WS-DTT}) and total ($OP^{Total-DTT}$) OP were assessed. The researchers operated on quartz and Teflon filters. A comparison of results obtained from different sites revealed only minimal differences in higher levels of OP^{WS-DTT} and $OP^{Total-DTT}$ near the roadside, showing that OP^{WS-DTT} and $OP^{Total-DTT}$ were spatially homogenous.

Microfluidics can be also employed to design online systems for measuring the DTT activity of ambient particles. Cobalt (II) phthalocyanine (CoPC)-modified carbon paste was employed by Sameenoi et al. [36] as working electrode material, this allows the selective detection of residual reduced DTT. The sensor was coupled directly to a Particle-into-Liquid-Sampler (PILS) system to measure aerosol oxidative activity in an online set-up. An array of working, reference, and auxiliary electrodes were included in a poly(dimethylsiloxane) (PDMS)-based microfluidic device. Offline validation of the electrochemical sensor was carried out by applying filter samples collected from urban areas and biomass burning events. Thereafter, online system performance was tested in the lab using standard reference urban dust and industrial incinerator ash aerosolized samples. The measured DTT activity was reported to have a good linear relationship with the aerosol concentration (R^2 from 0.86 to 0.97) with a time resolution of circa 3 min. This online system, based on a microfluidic electrochemical sensor was only validated in the lab.

Furthermore, Sameenoi et al. [54] developed a microfluidic paper-based analytical device (μPAD), which is inexpensive and provides fast analysis for measuring PM oxidative activity on filters collected by personal sampling. The oxidative activity measurement is based on the dithiothreitol assay (DTT assay), uses colorimetric detection, and can be completed in the field within 30 min following sample collection. The μPAD assay was validated against the traditional DTT assay using 13 extracted aerosols: The results showed no significant differences in the DTT consumption rate measured by the two methods. It must be highlighted that in this assay there were some difficulties in automation.

An online monitoring system directly tested in the field was developed by Eiguren-Fernandez et al. [37] (Figure 4). The authors used a liquid spot sampler (LSS), for particle collection, followed by the determination of DTT activity via light absorbance spectroscopy. The LSS used in the online monitor of the oxidative capacity of aerosols (o-MOCA) is a three-stage laminar-flow water condensation approach that allows the collection of particles as small as 5 nm into liquid. The flow rate of 3.0 L/min, selected for the sampling step, gave a good efficiency (95%). The DTT assay has been improved to allow an online time-resolved analysis of collected samples, and its performance evaluated using the 9,10-phenanthraquinone (PQ) as a standard redox-active compound. The measured detection limit for *o-MOCA* was 0.15 nmol/min. Laboratory testing showed a reproducible, linear response between the DTT consumption rate (nmol/min) and PQ concentration (μM) equal to 9.8 ± 1.2 nmol/min·μM PQ. Finally, the system was tested in the field for 3 days, with a 3 h time resolution. One of the advantages of this method is that it could be adapted to other collection methods or offline analysis of liquid extracts.
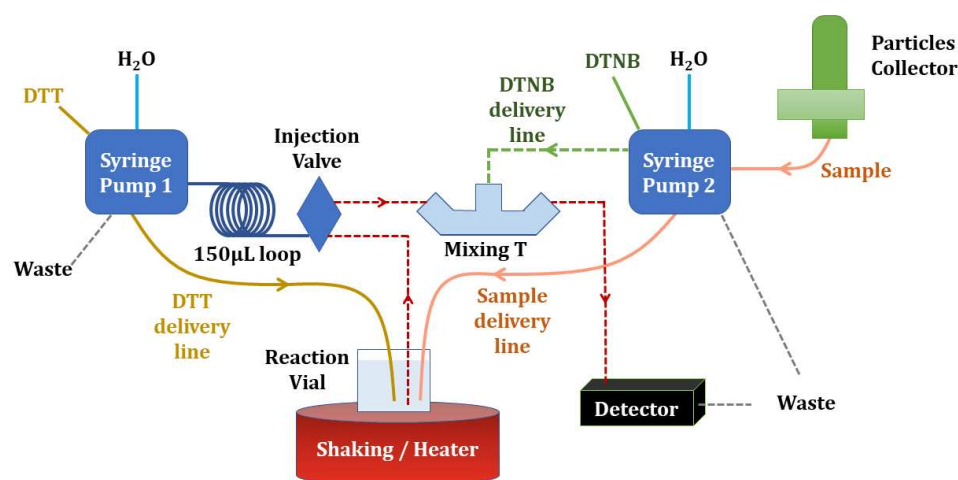

Figure 4. *o-MOCA* online system flow diagram, adapted from Eiguren-Fernandez et al. [37].

However, in the previously mentioned works including DTT analysis, the maximum number of continuous online measurement days on the field of 3 days (with 3 h time resolution) was reached only by Eiguren-Fernandez et al. [37]. This was a huge limitation for long-time PM monitoring in real time. Later, Puthussery et al. [40] investigated an increased number of days. In their work, they measured the hourly averaged OP of ambient $PM_{2.5}$ using DTT assay and its evaluation in the field conditions for over 50 consecutive days. They aimed to obtain long-term stability of the system to evaluate the DTT activity during all the time: In this case, data were obtained from 50 days of field deployment of the instrument. They developed a custom-built glass MC for collecting the ambient PM suspension, which was in turn transferred to an automated analytical system for DTT activity determination. The study reported for the first time, the complete diurnal profile of the DTT activity of ambient $PM_{2.5}$ at an hourly resolution. The OP_{ex} result obtained with the online instrument had a significant correlation ($R^2 > 0.8$; $p < 0.001$) with the data measured using the conventional filter sample and extraction (in water and methanol) methods. The instrument was tested in the field for 50 days with minimal manual assistance and the LOD tested was 0.24 nmol/min. Furthermore, around 65% of the hourly DTT activity measurements during field sampling were above the LOD. The diurnal variations in DTT activity were compared with the diurnal variations in $PM_{2.5}$ chemical composition. The outcomes indicated the importance of collecting excellent time-resolved data on $PM_{2.5}$ OP from various emission sources, as well as their relative importance in estimating the risk of $PM_{2.5}$ exposure throughout the day.

Furthermore, Puthussery et al., conducted field studies of the monitoring system developed in 2018 [40], first in 2020 [31] and then in 2022 [55]. The first field study

lasted around 1 week in Delhi, with highly time-resolved data (1 h resolution). The research presents the use of real-time online sensors in detecting distinct aerosol components and their emission sources that contribute to the OP of ambient $PM_{2.5}$. Despite limited measurements, precisely time-resolved data were collected, and authors observed statistically significant relationships with important chemical species. The comparison of the OPex versus aerosol mass measured at Delhi (OPex = 1.57 ± 0.7 nmol/min·m^3; average NR-PM1 = 105 ± 60 µg/m^3) with the typical levels observed in the earlier study in Illinois (OPex = 0.33 ± 0.19 nmol/min·m^3; average $PM_{2.5}$ concentration of 8 ± 0.25 µg/m^3) [40] clearly shows a no proportional relationship between aerosol mass concentration and extrinsic DTT activity. The outcome is consistent with emerging evidence of spatial heterogeneity in ambient $PM_{2.5}$ related to health response functions. In the second study [55], the real-time hourly averaged OP (based on a dithiothreitol assay) and $PM_{2.5}$ chemical composition were measured intermittently from October 2019 to January 2020. The results revealed the influence of biomass burning (BURN), Diwali fireworks, and fog events on the ambient fine particulate matter ($PM_{2.5}$) oxidative potential during the post monsoon (PMON) and winter seasons in Delhi, India. The peak extrinsic OP (OPv: normalized by the volume of air) was observed during the winter fog (WFOG) (5.23 ± 4.6 nmol/min·m^3), whereas the intrinsic OP (OPm; normalized by the $PM_{2.5}$ mass) was the highest during the Diwali firework-influenced period (29.4 ± 18.48 pmol/min·µg). Source apportionment analysis using positive matrix factorization revealed that traffic + resuspended dust-related emissions (39%) and secondary sulphate + oxidized organic aerosols (38%) were driving the OPv during the PMON period, whereas BURN aerosols dominated (37%) the OPv during the WFOG period. Firework-related emissions became a significant contributor (~32%) to the OPv during the Diwali period (4-day period from October 26 to 29), and its contribution peaked (72%) on the night of Diwali. Collectively, these results suggest that firework-related emissions could pose serious acute health risks, being the major contributors to both PM2.5 mass and OPv during the Diwali period. Results highlight the importance of real-time measurement of aerosol chemical composition and OP, in discerning the temporal trends of the contribution of various emission sources to the oxidative properties and the potential health effects of ambient $PM_{2.5}$.

In 2020 Berg et al. [35] introduced and evaluated a semi-automated DTT assay, which matches a traditional HPLC with either UV/vis absorbance or electrochemical detection. Commercial and custom-made electrochemical detectors were also compared before measuring ambient $PM_{2.5}$ filter samples. The optimized, semi-automated assay could process six samples per hour (83%-time savings compared to manual analysis). The electrochemical detection saved 40% on consumables cost compared to UV/vis detection. Moreover, the electrochemical cell is economical (less than 1$) and the electrodes are reusable for around 30 injections. The UV/vis and electrochemical detection sensitivities were reported to be 0.150 mAU·min/µM DTT and 0.218 Ip/µM DTT, respectively. The calibration curve revealed DTT detection linearity, sensitivity, and accuracy, with a coefficient of determination (R^2) of 0.991. This liquid-handling automation was considered suitable for a variety of autosamplers in other laboratories for DTT assay semi-automation.

An electrochemical Glassy carbon sensor, modified with gold nanoparticles, for the detection of DTT was developed by Romano et al., 2022 [56]. Glassy carbon electrodes, both classic (GC) and screen printed (SPE), were electrochemically modified with gold nanoparticles (AuNPs) to improve the sensitivity of the sensor. The authors established a deposition method that produces a stable and efficient modified surface in a reasonably easy and repeatable manner. Data collected from these experiments showed good electroanalytical performances in terms of high sensitivity (0.0622 µA cm^{-2} µM^{-1} and 0.0281 µA cm^{-2} µM^{-1}), low detection limit (0.750 µM and 1.5 µM), wide linear and dynamic ranges ranged over 2–4 orders of magnitude, the response time (few s) and reproducibility of the method. Although the method was not used in field, it was applied to assess OP for six PM from filter extracted samples and validated using the traditional spectrophotometric method.

6. Conclusions and Future Perspectives

Studies on the influence of particulate matter on human health are currently focusing on novel detection techniques, mainly for particles with small sizes (i.e., <1 μm) and related toxicity. The need for a suitable and easy method to measure, on a routine basis, critical chemical intermediates in biological and environmental studies, such as ROS, has resulted in a series of research studies aiming to develop automated systems of analysis. Automation has several advantages over manual processes and data collection, such as increased productivity, reliable results, reduced lab space needed, and less hands-on time. On the other hand, online measurements give fast results with the possibility of monitoring the data in, speeding up the evaluation process and the data analysis. Although some of the presented techniques are neither easy nor affordable, they are essential for the development process, as they aim to reduce labour-intensive and time-consuming operations while enabling rapid analysis. Many of these devices were implemented in the field, which is a crucial point for real-time measurements; however, there are still important limitations to consider. While several devices can collect samples in the field, significant disadvantages are represented by the rapidity, portability, robustness, and simplicity of the obtained PM OP measurement. Therefore, these studies highlight the need for novel approaches to assess rapid OP evaluation. With regard to the standard molecular probes employed, even though DTT and DCFH methods are comparable in terms of analytical speed (Tables 1 and 2), a huge limit in the DCFH assay is still represented by the type of detection method used, which is often spectrophotometry. Spectrophotometric analysis is one the most used techniques to detect changes in the concentration of a specific molecule. Despite its high sensitivity, this technology is time consuming, difficult to integrate into portable devices, and has substantial throughput limitations [52]. On the other hand, the use of the DTT assay, which has reported a lower limit of detection (2 pmol DTT/min, as stated by [53]), compared to DCFH (gas-phase ROS: 160 pmol H_2O_2/m^3; particle-phase ROS: 120 pmol H_2O_2/m^3), allow for the use of electrochemical or colorimetric detection methods, as well as other approaches such as AA, glutathione, and cysteine assays, which are faster and easily scalable techniques. It would be interesting to assess the multiple compositions of PM in parallel, using these molecules to develop effective predictor systems of health impacts. For instance, a microfluidic electrochemical device would offer a wide range of information on the OP state by running the reactions of one sample in various channels, each with a different probe, while employing small sample volumes. Therefore, the use of versatile methods enabling the development of miniaturising detection system while ensuring accuracy and robustness may be advantageous, especially when employing this technology in on-field analysis.

In conclusion, although the detection methods described above have certain limitations, the development of online and automated systems for OP measurement of PM is attractive and promising. The use of alternative probes in addition to better and miniaturized detection technologies may be beneficial in the development of portable devices, improving air quality monitoring in limited laboratory settings as well as on-field measurements.

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