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Enhancing DTT assays for reactive oxygen species detection in atmospheric particulate matter: key factors and methodological insights

W. P. D. Vimukthi, Shenghong Dong, Chi Yang, Yanlin Zhang, Gulinasahan Baikeri, Ting Lou, Fuyang Deng, Ziyang Li and Fang Cao*

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Accurate detection of reactive oxygen species (ROS) in atmospheric particulate matter (PM) is essential for assessing the oxidative potential (OP) of airborne pollutants and their associated health risks. While multiple methods exist for ROS detection, inconsistencies in assay conditions often lead to variable outcomes, limiting cross-study comparability. This review systematically evaluates key methodological factors affecting the dithiothreitol (DTT) assay, one of the most widely used techniques for measuring the OP of PM. Critical parameters—including intrinsic assay variables such as initial DTT concentration and incubation conditions, as well as extrinsic factors such as light exposure and metal–organic interactions—are analyzed to identify sources of variability. To improve sensitivity and reliability, this study proposes standardized protocols, the incorporation of positive controls, and methodological refinements. By addressing these challenges, this review enhances the accuracy of ROS detection and contributes to a more comprehensive understanding of the OP of PM, with significant implications for environmental monitoring and public health.

Environmental significance

This review highlights the critical role of reactive oxygen species (ROS) in determining the oxidative potential (OP) of particulate matter (PM), a key indicator of air pollution toxicity. By identifying and addressing methodological inconsistencies in ROS detection assays, this work underscores the need for standardized protocols to ensure accurate assessments of air pollution's health impacts. These insights contribute to advancing atmospheric science, improving air quality standards, and guiding targeted regulatory measures to mitigate oxidative stress-related health risks and environmental damage.

1. Introduction

Atmospheric Particulate Matter (PM) poses significant risks to both human health and environmental systems, primarily due to its ability to generate ROS, which contribute to oxidative stress and tissue damage.[1,2] Exposure to ROS from PM has been linked to various adverse health outcomes, including respiratory and cardiovascular diseases, due to its role in inducing cellular damage and inflammation.[3–5] Beyond health effects, ROS also play a critical role in atmospheric chemistry, particularly in the formation of secondary organic aerosols (SOAs), which contribute to air quality degradation.[6,7] Accurate detection of ROS is essential for assessing the OP of PM and understanding its broader health and environmental implications.

Various analytical techniques have been developed to measure ROS in PM, with the DTT assay emerging as one of the most widely used methods.[8–10] The DTT assay provides a quantitative measure of the oxidative potential of PM by assessing its capacity to deplete DTT, a thiol-based reducing agent. This method is favored due to its simplicity, cost-effectiveness, and reproducibility. However, significant variations in experimental protocols—such as differences in initial DTT concentration, incubation conditions, light exposure, and metal–organic interactions—compromise its reliability and hinder cross-study comparisons.[11,12] Understanding these inconsistencies is essential for improving the accuracy and standardization of ROS detection.[13–16]

The OP measured by the DTT assay is influenced by multiple PM components, including soluble metals (*e.g.*, copper and manganese)[14,17] and organic compounds like water-soluble organic carbon (WSOC),[18,19] oxidized polycyclic aromatic hydrocarbons (PAHs) such as quinones,[20,21] and humic-like substances (HULIS).[22,23] While these components strongly correlate with DTT oxidation, their interactions introduce complexities that can obscure causal relationships.[24–27] For instance, recent research suggests that metal ions not only drive direct oxidative reactions but also interact with organic compounds, further complicating ROS generation mechanisms.[7,18,28]

Despite its widespread use, the DTT assay presents several methodological challenges. The OP of PM can be expressed in

School of Ecology and Applied Meteorology, Nanjing University of Information Science and Technology, Nanjing 210044, China. E-mail: caofang@nuist.edu.cn; caofangle@163.com

two formats: volume-normalized DTT activity (DTTv), which is relevant for human exposure assessments, and mass-normalized DTT activity (DTTm), which provides insight into the intrinsic oxidative properties of PM components.[29,30] However, achieving reliable ROS measurements can be challenging due to practical factors like light exposure and the complex chemical nature of PM as illustrated in Fig. 1.

Unlike previous reviews, this study systematically evaluates key methodological factors affecting the DTT assay and proposes improvements to enhance its sensitivity and accuracy. By identifying sources of variability—such as light-induced ROS formation and metal–organic interactions—and recommending standardized protocols and the inclusion of positive controls, this review aims to improve the reliability of ROS detection. These refinements will contribute to a more accurate assessment of the oxidative potential of PM, advancing both environmental monitoring and health risk evaluation strategies.

2. Overview of ROS detection techniques for particulate matter

The detection of ROS in PM is essential for evaluating the OP of atmospheric pollutants. ROS detection techniques can be broadly classified into fluorescence-based techniques and spectrometric methods, each with distinct advantages and limitations, as summarized in Table 1.[39–41] Although multiple techniques exist, this review primarily focuses on the DTT assay, while briefly discussing alternative ROS detection methods to provide context.

2.1. Fluorescence-based techniques

Fluorescence-based methods are widely used for detecting particle-bound ROS due to their high sensitivity, rapid response, and adaptability. These techniques utilize chemical probes that emit fluorescence upon reacting with ROS, enabling quantitative analysis.[42,43] The most commonly used probe is dichlorofluorescin (DCFH), a non-fluorescent molecule that becomes fluorescent (DCF) upon oxidation by ROS. The DCFH assay involves mixing DCFH with horseradish peroxidase (HRP) in a sodium phosphate buffer, followed by sonication to extract ROS from PM samples. The fluorescence intensity is then measured and converted to hydrogen peroxide (H_2O_2) concentration using a calibration curve.[44–46]

Alternative particle-bound ROS detection techniques vary in reagents, concentrations, mixing procedures, and fluorescence measurement approaches. Commonly used reagents include 9-(1,1,3,3,tetramethylisoindolin-2-yloxyl-5-ethynyl)-10-

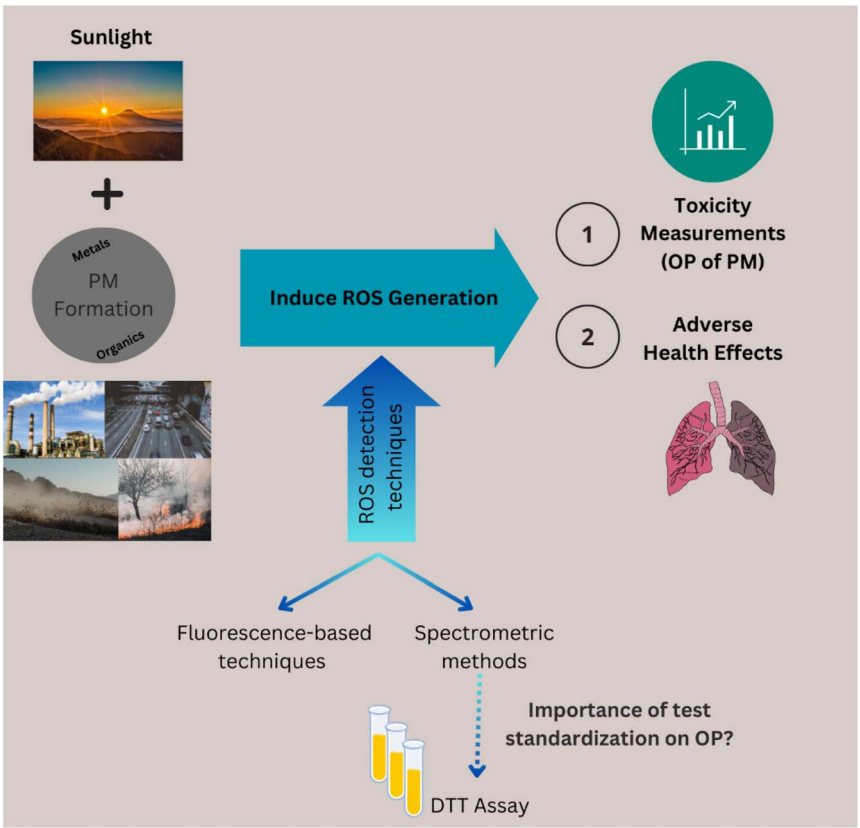

Fig. 1 Sources of PM, ROS detection techniques, and associated health impacts. Formation of PM involves contributions from metals, organic compounds, and sunlight, which collectively induce ROS generation. Detection of ROS, a key indicator of the oxidative potential of PM, is achieved using fluorescence-based and spectrometric methods, with the DTT assay as a common approach. The standardization of the DTT assay is critical to improve the reliability of OP measurements. Accurate assessment of OP is essential for understanding PM toxicity and its role in adverse health effects, emphasizing the importance of methodological consistency across studies.

Table 1 Comparison of ROS detection techniques in PM analysis. Summary of fluorescence-based and spectrometric techniques for detecting ROS in PM, highlighting each method's key advantages, limitations, and primary applications

Method	Advantages	Limitations	Reference
DCFH (dichlorofluorescein) assay	High sensitivity; rapid detection; adaptability	Cross-reactivity with multiple ROS types; sensitivity to pH and reagent concentration	31–33
DTT assay (chemical OP assay)	Quantitative OP measurement; established protocols	Sensitivity to metal ions and reaction conditions	17,34–36
EPR spectroscopy	Specific ROS identification	Requires specialized equipment and stabilization agents	37
Chemiluminescence techniques	High sensitivity for specific applications	Limited use in PM studies; specialized reagents required	38

(phenylethynyl)anthracene (BPEAnit), aminophenyl fluorescamine (APF), and 10-acetyl-3,7-dihydroxyphenoxazine (Amplex Red). APF is selective for hydroxyl radicals, while Amplex Red is primarily used for detecting H_2O_2.[47–49] However, fluorescence techniques can be limited by cross-reactivity with multiple ROS types, which can reduce specificity.[49] Additionally, the efficacy of these methods is influenced by experimental factors, such as pH, reagent concentration, and extraction methods.[50]

Thus, while fluorescence techniques provide a sensitive approach for ROS detection, their reliability depends on the careful selection of probes and optimized experimental conditions.

2.2. Spectrometric methods

Spectrometric methods measure ROS by detecting changes in light absorption or emission, providing precise quantitative data.[51] These techniques include chemiluminescence, UV-visible spectrophotometry, and electron paramagnetic resonance (EPR) spectroscopy.[40,41]

2.2.1. DTT assay: A key spectrometric method. The DTT assay, one of the most widely used spectrometric methods, evaluates the OP of PM by measuring DTT depletion. In this assay, PM samples are incubated with DTT in a potassium phosphate buffer for a duration of 15 to 90 minutes. The remaining DTT is quantified spectrophotometrically after quenching the reaction with trichloroacetic acid (TCA) and reacting with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), which produces a measurable colored product.[8,52] The DTT assay is particularly sensitive to metal-driven oxidative reactions, as transition metals (*e.g.*, Fe, Cu, and Mn) can accelerate DTT depletion.[14]

2.2.2. Other spectrometric methods. Electron Paramagnetic Resonance (EPR) spectroscopy is a highly specific technique used to detect unpaired electrons in free radicals, such as superoxide (O_2^-) and hydroxyl radicals ($\cdot$OH). This method provides detailed information about radical species but typically requires the use of specialized spin-trapping agents to stabilize these highly reactive intermediates.[53,54] Another emerging technique is the Chemiluminescent Reductive Acridinium Triggering (CRAT) assay, which measures light emitted from reactions between oxidants and luminescent reagents. Although the CRAT assay shows promise for oxidative potential assessment, it has not yet been widely applied in studies involving PM.[38]

While spectrometric methods are precise and valuable for quantitative data, they are sensitive to factors like the choice of extraction solvent, incubation time, and presence of metal ions, which can complicate cross-study comparisons.[55–57]

2.3. Selecting an appropriate ROS detection method

Selecting an appropriate ROS detection method depends on study-specific factors, including the required sensitivity, specificity, equipment availability, and the intended level of ROS identification.[11,58,59] Among spectrometric methods, the DTT assay stands out for its reproducibility and applicability in PM research. While EPR spectroscopy offers high specificity for ROS analysis, its complexity and resource-intensive nature limit its routine use in OP assessments. Fluorescence-based techniques, although highly sensitive, primarily serve studies requiring rapid ROS detection rather than comprehensive OP analysis. Given these considerations, this review primarily focuses on the DTT assay as a robust and widely applied method for evaluating the OP of PM.

3. Key methodological considerations of the DTT assay in ROS detection

The DTT assay is widely used to measure the OP of PM by assessing its ability to deplete DTT, a thiol-based reducing agent. However, the reliability of this assay is influenced by several internal and external factors, including initial DTT concentration, incubation conditions, light exposure, and metal–organic interactions. Addressing these factors is crucial for improving assay consistency and cross-study comparability.

3.1. Internal assay variables and their influence on ROS detection

A clear understanding of how DTT functions as a reducing agent is essential for optimizing its use in OP measurements. Key factors such as initial DTT concentration, incubation conditions, and assay standardization significantly affect the reproducibility and accuracy of results.

3.1.1. Role of DTT as a reducing agent in measuring OP in ROS assays. The OP of PM is commonly assessed using

chemical assays such as the DTT assay, ascorbic acid (AA) assay, and glutathione (GSH) assay. The DTT assay is widely preferred due to its affordability, simplicity, and high repeatability. It quantifies the rate of DTT consumption as an indicator of ROS generation, particularly for O_2^- and H_2O_2.[11,26,36,60,61] Cho *et al.* (2005) first introduced the DTT-based approach for OP evaluation, making it a widely accepted tool.[8]

Fig. 2 illustrates the two-step process involved in the DTT assay, a method for assessing the OP of PM. In Step A, DTT functions as a reducing agent, mimicking the production of ROS by transferring electrons from DTT to oxygen, which leads to the formation of superoxide anions and hydrogen peroxide. This reaction is driven by redox-active compounds in PM, such as quinones, and can generate additional ·OH in the presence of transition metals like Fe and Cu through Fenton-like reactions. During this process, DTT is converted to its disulfide form (DTT-disulfide).[10,13,14,60]

In Step B, any remaining DTT that hasn't reacted yet quickly binds with DTNB, producing DTT-disulfide and a yellow compound called TNB. TNB has a strong molar extinction coefficient of 14 150 M^{-1} cm^{-1} at 412 nm, which makes it measurable by UV-visible spectrophotometry to determine the rate at which DTT is consumed—indicating the oxidative potential of the PM.[10,60] The DTT consumption rate (σDTT), is calculated from the linear slope of DTT depletion and serves as an OP indicator. Essentially, the rate of ROS production by a PM sample is inferred from the DTT consumption rate, which is directly proportional to the concentration of redox-active compounds in the sample.[8,11,13,64] Eqn (1) expresses the rate of DTT consumption in nmol min^{-1}.

$$\sigma\mathrm{DTT}_{\text{blank or sample}} = -\sigma\mathrm{Abs}\ \frac{N_0}{\mathrm{Abs}_0} \quad (1)$$

where, $-\sigma$Abs = the slope of absorbance *versus* time, Abs_0 = the absorbance calculated from the intercept of the linear regression *versus* time, and N_0 = the initial moles of DTT added into the reaction vial (nmol).

In the literature, DTT oxidative potential ($\mathrm{OP}_{\mathrm{DTT}}$) is typically reported using two standard units. The first, DTTv (nmol DTT min^{-1} m^{-3}), measures the rate of DTT consumption per minute per unit volume of sampled air, making it particularly relevant for evaluating human exposure. The second, DTTm (nmol DTT min^{-1} μg^{-1}), normalizes the DTT consumption rate by the mass of particulate matter in the reaction, providing insight into the particulate matter's intrinsic oxidative potential, as shown in eqn (2) and (3).[65–67]

$$\mathrm{DTTv} = \frac{r_s\ (\mathrm{nmol\ min^{-1}}) - r_b\ (\mathrm{nmol\ min^{-1}})}{V_t\ (\mathrm{m^3}) \times \frac{A_h\ (\mathrm{cm^2})}{A_t\ (\mathrm{cm^2})} \times \frac{V_s\ (\mathrm{mL})}{V_e\ (\mathrm{mL})}} \quad (2)$$

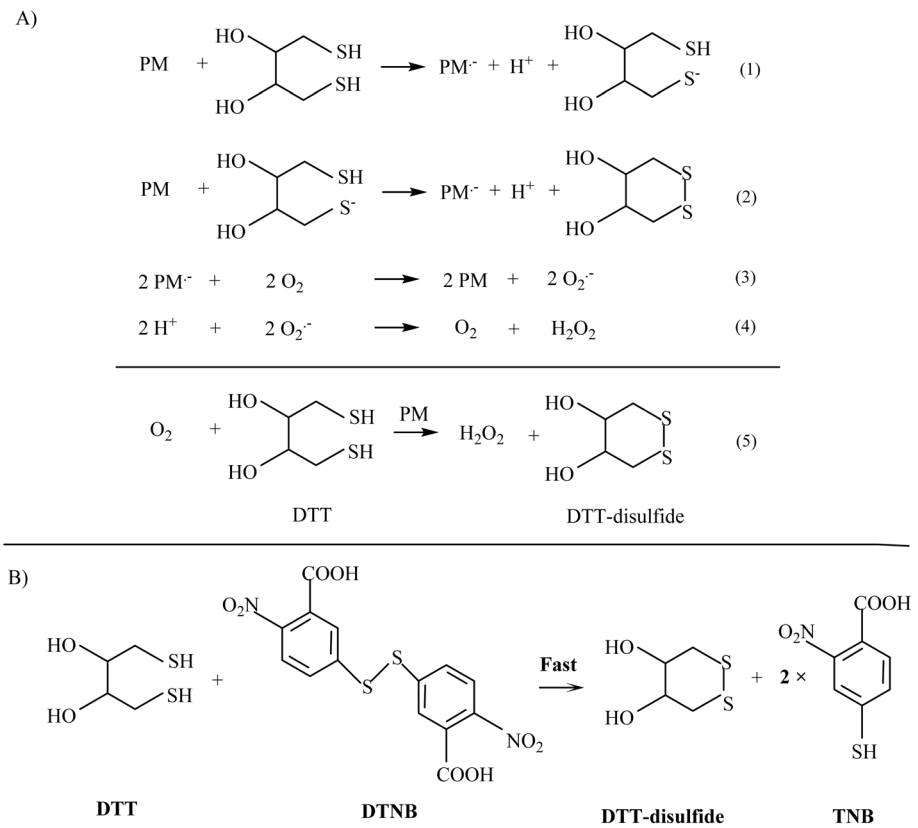

Fig. 2 (A) Reduction of O_2 by DTT, leading to the formation of ROS, with PM acting as a catalyst. (B) Reaction of DTT with DTNB, resulting in the formation of the colored product 2-nitro-5-thiobenzoic acid (TNB), which is measured spectrophotometrically. Adapted from Ayres *et al.*, 2008;[62] Rattanavaraha *et al.*, 2011;[27] Visentin., 2016;[63] and Thomson 2022.[60]

$$\mathrm{DTTm} = \frac{r_s\ (\mathrm{nmol\ min^{-1}}) - r_b\ (\mathrm{nmol\ min^{-1}})}{M_t\ (\mu\mathrm{g}) \times \frac{A_h\ (\mathrm{cm^2})}{A_t\ (\mathrm{cm^2})} \times \frac{V_s\ (\mathrm{mL})}{V_e(\mathrm{mL})}} \quad (3)$$

where r_s and r_b are the DTT consumption rates of the sample and blank, respectively. V_t and M_t are the total sampling air volume and the total particle mass (with filter blank correction), respectively. A_h and A_t are the area of the hole and total filter, respectively. V_s and V_e are the sample volumes participating in reaction and extraction volume, respectively.

The DTT assay is widely applied in aerosol studies, including both primary and secondary organic aerosols (SOAs).[6,11,12,26] However, non-catalytic pathways also contribute to total OP measurements, requiring careful interpretation.[11,26,36]

3.1.2. Optimal initial DTT concentration and incubation conditions. Optimizing initial DTT concentration and incubation conditions is crucial for precise ROS detection. Studies indicate that DTT consumption rates are proportional to initial concentrations,[12] with typically use levels ranging from 20 μM (ref. 68 and 69) to 100 μM.[14,70]

Setting optimal incubation conditions for DTT assays also presents challenges, as studies vary widely in their approach. Extracted PM samples are usually incubated with buffer solutions (pH 7–7.4) and DTT at 37 °C for a set period, after which the reaction is halted by adding TCA. While some studies use a fixed incubation time (30–60 minutes) before measuring absorbance to quantify DTT consumption,[10] others continuously monitor DTT depletion at multiple points to calculate the slope and intercept, giving a more accurate measurement of the DTT depletion rate.[11,34,71–73] Evidence suggests that DTT consumption may not remain linear throughout the incubation, and using a fitted slope can provide a more reliable metric for OP.[12,13] However, some samples reach a plateau or show reduced DTT consumption over time, likely due to the depletion of catalytically active species, interference from DTT-inhibiting compounds, or secondary reactions.[8]

To ensure accuracy, it's essential to measure DTT consumption within the linear range, which depends on the sample's composition and incubation conditions.[11] Charrier and Anastasio recommend limiting incubation times to this range to avoid inaccuracies, especially when non-catalytic DTT-reactive species are present. Keeping DTT loss within 20% of its initial concentration helps maintain linearity, and assays are often conducted under pseudo-first-order conditions, where less than 50% of DTT is consumed during the reaction.[14] Proper control of initial DTT concentration and incubation conditions is crucial to avoid bias. A summary of methods used in past studies is presented in Table 2. The variability in OP values across studies highlights the need for standardized protocols to improve the consistency and comparability of results[11] (see Section 3.1.3).

3.1.3. Standardization issues and refinements. Despite its widespread use, the DTT assay lacks standardized protocols, affecting its reproducibility. Key variables—such as incubation temperature, light exposure, mixing methods, and chelating agents like ethylenediaminetetraacetic acid (EDTA)—can influence ROS detection outcomes. Table 3 outlines critical factors affecting assay performance and the corresponding controls to enhance accuracy and comparability.[11]

While previous reviews, including that of Jiang *et al.* (2020), have highlighted the need for standardization in the DTT assay, this study builds upon their framework by incorporating specific methodological refinements. For instance, controlling light exposure to prevent photo-oxidation is critical, and using amber glassware for DTT and DTNB storage can mitigate this risk. Additionally, maintaining an incubation temperature of 37 °C ensures consistency in DTT consumption, aligning with physiologically relevant conditions.

A key contribution of this study is the introduction of positive controls, such as ferro-ammonium oxalate (FAO)[11] and quinones like 1,4-naphthoquinone,[60] to enhance assay validation. These controls mimic the redox cycling of ROS under ambient conditions, making them particularly suitable for air quality studies. The inclusion of quinones, which naturally occur in atmospheric PM, strengthens the environmental relevance of the assay and adds a novel dimension to its standardization.

Incorporating these standardization practices, alongside the use of tailored positive controls, will significantly enhance the reproducibility and utility of the DTT assay in environmental and health-related research. These improvements will lead to a more reliable understanding of the oxidative potential of particulate matter and its implications for human health and atmospheric processes.

3.1.4. Detecting a range of ROS with DTT assay: challenges and limitations. The DTT assay primarily detects ROS linked to H_2O_2 production but is less effective for other species, such as $\cdot OH$ or O_2^-. To obtain a comprehensive assessment of PM OP, researchers recommend combining multiple acellular assays.[11,61,78] For example, disodium terephthalate (TPT) can be used to detect $\cdot OH$,[61,72] while the luminophore coelenterazine serves as a probe for O_2^-,[79,80] providing broader insights into metal-induced ROS.[81] Additionally, techniques such as EPR or electron spin resonance (ESR) are useful for identifying short-lived free radicals that the DTT assay alone cannot detect. Using complementary techniques provides a broader understanding of PM oxidative activity and its potential health risks.[11]

Additionally, PM components such as transition metals and organic compounds can enhance or inhibit ROS generation through catalytic or non-catalytic pathways. Transition metals, for instance, can catalyze reactions that lead to higher ROS levels, thus impacting the measured OP. Organic particulates, especially highly oxidized compounds, may interact with DTT and complicate result interpretation. The following section examines external factors on ROS measurements, including light exposure and chemical influences.

3.2. Influence of external factors on ROS generation and detection

3.2.1. Influence of light exposure on ROS

3.2.1.1. Mechanism of ROS generation through photo-oxidation. Airborne PM can undergo photo-oxidation reactions, which play a major role in ROS generation.[6,82,83] These reactions involve

Table 2 Summary of various DTT assay methods reported previously in the literature[a]

$[DTT]_0$ (μM)	Method	Withdrawal (mL)	Incubation time (min)	Reaction time (min)	Detection instrument	Reference	Primary findings/effectiveness
100	37 °C, incubator	0.5	15–90	Variable (unspecified)	UV-vis spectrophotometer	(Cho *et al.*, 2005)[8]	Requires linear rate of DTT consumption; % CV <15% for accuracy
20	37 °C, incubator	—	30	Fixed with incubation period	UV-vis spectrophotometer	(Li *et al.*, 2009)[10]	Fast DTT-DTNB reaction; no quenching needed; simplifies the procedure
100	37 °C, dry bath	0.5	Variable (unspecified)	Variable (unspecified)	UV-vis spectrophotometer	(Charrier and Anastasio, 2012)[14]	Followed the DTT assay method of Cho *et al.* (2005); improved stability with Chelex 100 resin; DTT loss <20%; EDTA is not recommended in DTT assay
100	37 °C, incubator, shaken continuously	0.1	0, 4, 13, 23, 32, 41	Fixed with incubation period	LWCC + UV-vis spectrometer	(V. Verma *et al.*, 2014)[74]	Followed the DTT assay method of Cho *et al.* (2005); automated system; consistent results; analyses one sample per hour
100	37 °C, incubator, shaken continuously	0.1	4, 13, 23, 30, 41	Fixed with incubation period	LWCC + UV-vis spectrometer	(T. Fang *et al.*, 2015)[13]	Semi-automated system; good agreement with manual methods; seasonal variability
20	37 °C, dry bath	0.1	90	Fixed with incubation period	UV-vis spectrophotometer	(Y. Ma *et al.*, 2018)[75]	Followed the DTT assay method of Li *et al.* (2009) and Lin and Yu (2011); DTT activity linearly proportional to HULIS WS mass concentration
100	37 °C, incubator	0.1	3, 15, 27, 39, 51	Fixed with incubation period	LWCC + UV-vis spectrometer	(Yu *et al.*, 2018)[72]	Measured DTT consumption and OH generation; interactions of metals with DTT
100	37 °C, incubator	0.5	0, 10, 20, 30	Fixed with incubation period	UV-vis spectrometer	(J. Wang *et al.*, 2019)[36]	DTT activity varies diurnally and seasonally; important for exposure level studies
100	37 °C, water bath	0.2	0, 5, 10, 20, 30, 45, 60	Fixed with incubation period	Microplate reader (visible light)	(Lu *et al.*, 2019)[67]	DTT oxidation results correlated with ROS levels, seasonal variations observed
100	37 °C, incubator	0.2	0–30 (every 10 min)	Fixed with incubation period	UV-vis spectrometer	(Lin and Yu, 2019)[12]	Metal concentration affects DTT consumption rates differently; emphasized importance of standardized DTT assay methods for accurate comparisons
100	37 °C, incubator	0.1	Variable	Fixed with incubation period	UV-vis spectrophotometer	(H. Jiang *et al.*, 2020)[26]	DTT consumption rate increases with initial concentrations; second-order reaction with non-catalytic species
100	37 °C, incubator	0.1	0, 4, 13, 23, 31, 41	Fixed with incubation period	Online spectrometer	(D. Gao *et al.*, 2020)[2]	DTT assay measures water-soluble and total oxidative potential; seasonal effects

Table 2 (*Contd.*)

$[DTT]_0$ (μM)	Method	Withdrawal (mL)	Incubation time (min)	Reaction time (min)	Detection instrument	Reference	Primary findings/effectiveness
100	37 °C, water bath	0.1	0, 4, 13, 23, 32, 41	Fixed with incubation period	UV-vis spectrophotometer	(Y. Wang, *et al.*, 2020)[76]	Seasonal DTTv levels highest in winter; long-range transport impacts oxidation
100	37 °C, water bath	1	0, 15, 30, 45, 60	Fixed with incubation period	Multifunctional microplate reader	(Ma *et al.*, 2021)[77]	Seasonal variations in oxidative potential; transition metals impact winter months
1000	37 °C, incubator	1	0, 10, 20, 30, 40	Fixed with incubation period	LWCC	(Wu *et al.*, 2022)[64]	Improved online ROS analyzer; optimized system reduced DTT consumption rate by 44%; daytime ROS higher

[a] $[DTT]_0$ is the initial DTT concentration in the reaction tube in μM.

interactions between organic compounds,[6] transition metals,[83] and molecular oxygen (O_2) with ultraviolet (UV) light exposure acting as a primary driver.[84] UV radiation triggers complex photochemical reactions in organic aerosols (OAs), where organic molecules absorb light, causing their electrons to reach higher energy states. In these excited states, the organic compounds can react with O_2, leading to the formation of ROS like singlet oxygen ($^1O_2$), O_2^-, and H_2O_2, as highlighted by Jiang and Jang.[6]

Additionally, photo-oxidation of specific aromatic compounds (ACs) including catechol (CAT), phthalic acid (PA), and 4,4′-oxydibenzoic acid (4,4′-OBA) has been shown to significantly enhance ROS production, as demonstrated in a study by Hu *et al.*, 2023. Their research found that longer light exposure increases ROS concentration, often following zero-order reaction kinetics for certain ACs.[82] This light-induced ROS generation substantially contributes to the oxidative capacity of atmospheric PM, influencing both environmental processes and human health.

Research by Jiang and Jang also highlights the impact of photo-oxidation of iron species in PM on ROS generation. Sunlight drives the photochemical reduction of Fe(III) to Fe(II), which readily participates in Fenton reactions, generating highly reactive ·OH when combined with H_2O_2. This transformation is more active during daylight hours due to sunlight exposure, leading to higher daytime ROS production compared to nighttime.[83]

3.2.1.2. Role of light exposure in ROS detection. The detection of ROS, particularly through the DTT assay, is significantly impacted by light exposure.[6,85] The DTT assay is commonly used to measure the oxidative potential of PM by assessing the rate of DTT consumption, which indicates ROS generation.[11,13,14,64] Research by Steven Thomson (2022) has shown that the photodegradation of chemicals involved in the DTT assay leads to higher DTT depletion rates. Samples shielded from light displayed a much lower DTT depletion rate (0.385 ± 0.014 nmol DTT min^{-1}) compared to samples exposed to light (0.714 ± 0.34 nmol DTT min^{-1}). To address this issue, researchers recommend using amber glass bottles and flasks for storing and preparing DTT and DTNB solutions.[60]

Under photo-oxidative conditions, the generation of ROS from aromatic compounds (ACs) also increases. Hu *et al.* found that ROS concentration from catechol (CAT) and phthalic acid (PA) gradually rises with extended light exposure, whereas 4,4′-oxydibenzoic acid (4,4′-OBA) maintains a more stable ROS concentration after an initial spike.[82] This suggests that the photoreaction products formed during light exposure are more effective in inducing ROS than the original compounds, emphasizing the need to factor in light exposure duration in ROS detection methods.

Additionally, light exposure influences the OP of OAs, particularly during their aging process.[6,85] Research by Jiang and Jang on freshly emitted wood smoke particles demonstrates a linear rise in DTT consumption over time, indicating that catalytic processes primarily drive the oxidative potential. However, prolonged sunlight exposure reduces the oxidative potential as particulate oxidizers, like quinones and organic hydroperoxides (OHP), begin to degrade, causing a shift from

Table 3 Key influencing factors affecting ROS detection in DTT assays

Factors	Impact on ROS detection	Recommended control
Light exposure	Enhances ROS generation through photo-oxidation, potentially leading to elevated DTT depletion rates[6]	Store DTT and DTNB solutions in amber glass and perform assays under controlled lighting conditions
Incubation temperature	Higher temperatures (*e.g.*, 37 °C) improve consistency in ROS detection[10]	Maintain the incubation temperature at 37 °C to align with physiologically relevant conditions
pH levels	ROS generation is pH-sensitive, with phosphate buffers at pH 7.4 offering optimized reaction conditions[14]	Use standardized buffer solutions (pH 7.4 for phosphate, pH 8.9 for tris) across studies
Incubation time	Prolonged incubation may result in non-linear DTT consumption, affecting OP values[26]	Limit the incubation time to 30–60 minutes, ensuring DTT consumption remains within a linear range
Mixing techniques	Ultrasonic water[77]/dry[14] baths or shaking incubators[8] can introduce variability in assay results	Standardize mixing techniques to avoid deviations in DTT consumption and assay outcomes
Chelating agents	Chelating agents like EDTA may suppress DTT activity by binding metal ions essential for the reaction, leading to an underestimation of ROS production[14]	As an alternative, Chelex resin can be used to remove trace metals without inhibiting DTT reactivity[12,76]
Standardized calibration	To ensure comparability across studies, it is critical to maintain a consistent initial DTT concentration, initial PM concentration, and reaction linearity	Maintain an initial DTT concentration of 0.1 mM,[8] limit PM concentrations to 5–20 μg mL^{-1},[11] and ensure reaction linearity by capping DTT consumption at less than 90%

catalytic to noncatalytic ROS generation processes. This effect is particularly observed in SOAs formed *via* photo-oxidation of hydrocarbons, where DTT consumption varies based on particle aging.[6]

Further laboratory studies, including those by Hu *et al.* (2023), have shown that UV irradiation can raise soluble Fe(II) levels in PM, leading to increased ROS concentrations. This underscores the critical role of light exposure in interpreting ROS detection outcomes, as oxidative processes under UV light can produce marked differences in ROS levels.[82] Recognizing the dynamics of light exposure is thus essential for accurately interpreting ROS data and ensuring that experimental conditions align with realistic atmospheric scenarios.

3.2.1.3. Effect of light on transition metals and organic compound interactions. The interaction between transition metals (TMs) and organic compounds under light exposure plays a pivotal role in ROS generation and detection in atmospheric aerosols.[83,86] Transition metals such as iron (Fe) and copper (Cu) are key in catalyzing ROS formation *via* Fenton-like reactions.[1] UV radiation enhances the redox cycling of these metals, promoting their interactions with organic compounds, which leads to the production of reactive ·OH from H_2O_2.[82,87,88] For instance, research by Xiaoyu Hu has shown that catechol (CAT) combined with Fe(II) and Cu(II) under light exposure has a synergistic effect that substantially boosts ROS generation. However, the interactions between aromatic compounds (ACs) and transition metals can also show antagonistic effects. For example, Mn(II) initially enhances ROS production but later stages show a decline, highlighting the complexity of these interactions.[82]

Additionally, the photo-oxidation of organic compounds releases reactive intermediates that further interact with transition metals, forming a network of reactions that impact the oxidative potential of PM. Organic ligands within PM can stabilize metal ions in their reduced states, aiding in the production of soluble Fe(II) under UV light.[1,6,27,83,89] Conversely, some organic compounds may scavenge ROS, potentially lowering sensitivity in assays like the DTT assay.[28] Chelating agents, such as diethylenetriaminepentaacetic acid (DTPA), influence these interactions, adding complexity to ROS detection.[6]

3.2.2. Chemical influences of PM on ROS generation. The complex chemistry of PM necessitates an understanding of metal ions, organic compounds, and their interactions within PM, as these factors significantly influence ROS formation and the oxidative potential of the sample. Chemical interactions play a key role in the reactivity and detectability of ROS.

3.2.2.1. Metal ions as catalysts and interferences in ROS detection. Metal ions are key catalysts in generating ROS, yet they can also interfere with various ROS detection methods[28]. Transition metals, particularly Fe and Cu, play a major role in ROS generation due to their redox cycling ability.[90] The Fenton reaction, in which H_2O_2 reacts with ferrous (Fe^{2+}) to produce highly reactive ·OH, is a key pathway for ROS formation in both environmental and biological contexts.[1,72] Other metals like manganese (Mn) and vanadium(v) also contribute significantly to ROS generation.[72,83]

The solubility of metal ions further influences their catalytic activity, as only soluble metals participate effectively in redox reactions, making metal solubility in PM a critical factor in evaluating the oxidative potential.[14] Environmental factors, such as pH, affect metal solubility, with acidic conditions increasing metal mobility and reactivity, thereby enhancing ROS production.[14,58] For instance, adding metal chelators to PM can significantly reduce ROS generation, underscoring the vital

role of soluble metal ions in the oxidative potential of PM across various assays.

While early research suggested that DTT oxidation was less sensitive to metals, recent findings reveal that transition metals—including Cu, Mn, and zinc (Zn)—significantly influence DTT depletion in ambient $PM_{2.5}$.[91] Zn though not redox-active, binds strongly to DTT, consuming it without undergoing redox cycling.[91] Numerous studies show correlations between DTT depletion and concentrations of Fe, Cu, Mn, and Zn, highlighting the central role these metals play in oxidative potential assays.[9,24] Consequently, metals, rather than organic species like quinones, often serve as the primary contributors to DTT oxidation in PM samples.

However, metal ions can also interfere with ROS measurement assays, such as the DTT assay. Metal contamination in reagents and samples may increase DTT depletion rates, leading to an overestimation of the oxidative potential. To reduce these interferences, chelation strategies, such as using EDTA or Chelex 100 resin, can remove trace metals from solutions.[14] It is essential to note, however, that while EDTA can reduce background DTT loss, it may also suppress responses from both metals and organic species, complicating the assessment of their contributions to ROS generation.[14]

3.2.2.2. Influence of organic species on ROS generation. The organic content of PM significantly influences ROS detection. Organic carbon (OC) and WSOC have been widely studied, with numerous global studies demonstrating a strong correlation between these components and OP_{DTT} across different seasons.[7,8,92] In the Los Angeles basin, 88% of the variability in volume-normalized OP_{DTT} for quasi-ultrafine ambient PM can be attributed to WSOC, water-insoluble organic carbon (WIOC), elemental carbon, and hopanes. Similarly, OAs contribute to 60% of the OP_{DTT} of water-soluble $PM_{2.5}$ in the southeastern United States, where the hydrophobic fractions of both water-soluble and water-insoluble organic aerosols significantly contribute to mass-normalized OP_{DTT}.[7,56,58,93]

PAHs, such as phenanthrene and pyrene, are major contributors to OP_{DTT}. Upon oxidation, these PAHs form DTT-active quinones, including phenanthraquinone (PQN), 1,2-naphthoquinone (1,2-NQ), and 1,4-naphthoquinone (1,4-NQ), with PQN being the most reactive, and participate in redox reactions that generate ROS, including O_2^- and H_2O_2.[8,14,20,21,61] These quinones can bind to soot particles, and black carbon coated with 1,4-NQ has been shown to significantly increase mass-normalized OP_{DTT} compared to untreated black carbon[21,94,95] Methanol extracts of PM often exhibit higher OP_{DTT} than water-soluble PM extracts, as methanol—a less polar solvent—extracts both hydrophilic and hydrophobic organic species. Nevertheless, water-soluble organic PM components remain essential contributors to OP_{DTT}, likely due to their greater biological availability.[7,27,55,56,58]

Interactions among organic species further influence OPDTT. Nitrogen-containing bases, such as pyridine and imidazole (commonly found in HULIS), enhance OPDTT in the presence of quinones by facilitating hydrogen atom transfer during ROS generation.[96,97] High molecular weight organic compounds with multiple reactive functional groups are also abundant in ambient PM and may impact DTT consumption, although their exact effects remain incompletely understood.[39,98] These observations underscore the importance of understanding the complex interactions between organic species and ROS detection probes to accurately assess the OP of PM.

Additionally, the chemical aging of organic species in PM enhances their redox activity. Photochemical aging, especially in urban environments where sunlight and atmospheric conditions promote SOA formation, converts less reactive organic compounds into more reactive forms like quinones.[58] Aged aerosols exhibit higher oxidative potential than fresh OA, highlighting the critical role of aging processes in determining the oxidative capacity of PM.[6,99] Certain organic compounds can undergo photochemical reactions in the presence of sunlight, leading to the generation of ROS such as H_2O_2 and organic peroxides.[6,82,83,100] Compounds like humic and fulvic acids can also interact with metal ions, affecting metal reactivity.[6] The presence of these organic species enhances ROS generation through various mechanisms, including the formation of metal–organic complexes.[11]

3.2.2.3. Effect of metal–organic complexes on ROS generation in PM. Metal–organic complexes in PM are essential in modulating ROS generation, either by enhancing or inhibiting ROS production through several mechanisms. These complexes can improve the solubility and reactivity of metal ions, making them more available for redox reactions that generate ROS.[1,18] For example, Wei *et al.* demonstrated that organic ligands stabilize metals in their more reactive reduced forms, like Fe(II), which participate in ROS production *via* Fenton-like reactions. In ambient PM, 70–90% of water-soluble Fe and Cu is often complexed with organic compounds, illustrating the considerable role these complexes play in driving ROS generation. Additionally, the interaction of Fe with organic species such as Suwannee River fulvic acid (SRFA) enhances the formation of ·OH, emphasizing the synergistic effects of metal–organic interactions on ROS production.[1]

Research also indicates that interactions between soluble metals—such as Fe, Cu, and Mn—and quinones can result in both synergistic and antagonistic effects in OP assays. For instance, Fe and Cu enhance the oxidative effects of quinones in assays measuring OP_{DTT} in surrogate lung fluid (SLF).[58,61,101,102] However, the impact of these interactions can vary depending on the specific metal–organic combination. Soluble Mn, for example, shows a synergistic effect with quinones in OP_{DTT} but an antagonistic effect on ·OH generation.[72] Similarly, Fe displays additive and synergistic effects with quinones in both DTT consumption and hydroxyl radical production, whereas Cu exhibits antagonistic effects in both processes.[72]

Furthermore, WSOC enhances the solubility of metals such as Fe through complexation, increasing the oxidative potential of PM, particularly in the presence of Fe.[18] Understanding these interactions is crucial for developing mitigation strategies that consider both total metal and organic concentrations as well as conditions that promote their interactions. Reducing such interactions could significantly lower the oxidative potential of PM in polluted areas, positively impacting public health.

Additionally, DTT itself can form stable complexes—both polymeric and monomeric—with metals such as Zn(II), Cd(II),

Pb(II), Ni(II), and Cu(I), adding complexity to DTT consumption in PM samples.[103] These interactions underscore the intricate role of metal–organic complexes in ROS modulation and highlight the need for further research to better understand these synergistic and antagonistic effects across different OP assays.

3.2.3.4. Practical implications and mitigation strategies. Understanding the role of metal–organic complexes in ROS generation has important implications for policy and pollution control. Identifying key contributors to ROS formation, such as metal–organic complexes, could help shape targeted strategies for reducing pollution. For example, emissions from sources that release both OA and transition metals—such as industrial operations and vehicle exhaust—could be managed more effectively to lower the oxidative potential of PM, thereby reducing related health risks.[58] Incorporating this understanding into air quality regulations could lead to standards that address not only individual pollutants but also the interactions that amplify ROS production and oxidative stress in exposed populations.

4. Conclusion and future directions

The DTT assay, a widely used method for assessing the OP of PM, faces challenges in standardization and is influenced by external variables such as light exposure and metal–organic interactions. To advance ROS detection in environmental and health research, focused efforts are needed in several key areas:

• Standardization of protocols: establishing standardized procedures for DTT assays, including consistent initial DTT concentration, PM mass concentration, incubation time, and avoiding the use of EDTA—will reduce variability in ROS measurements. Standardization would enable accurate comparisons across studies, enhancing the consistency of OP data and making the DTT assay more adaptable to real-world environmental monitoring.

• Optimization of detection methods: future research should prioritize refining and enhancing the specificity of both fluorescence-based and spectrometric ROS detection methods. Improving probe selectivity for distinct ROS types (*e.g.*, ·OH, H_2O_2) will increase measurement precision across varied environmental conditions and pollution profiles.

•ROS generation through photo-oxidation: light exposure, particularly under UV and sunlight, significantly drives ROS generation *via* photo-oxidative processes. Future studies could examine the effects of photo-oxidative mechanisms on ROS production in PM under different atmospheric conditions, such as high-oxygen *versus* anaerobic environments, to mirror real-world conditions. Standardizing procedures for managing light exposure during ROS measurements will improve data accuracy and deepen our understanding of how sunlight impacts the oxidative potential in different geographies and seasons.

• Impact of metal–organic interactions: the interaction of metal ions with organic compounds in PM is central to understanding ROS formation. Investigating these interactions under different emission scenarios (*e.g.*, areas with high metal emissions or organic pollutants) could provide insights into how specific sources contribute to oxidative stress in the environment.

This review underscores the importance of refining DTT assays to enhance the consistency and reliability of oxidative potential measurements. By integrating these standardized approaches into public health frameworks, researchers can better assess PM toxicity and mitigate associated risks.

5. Policy and public health implications

The insights derived from this review have significant implications for both public health and air quality management. Improved ROS detection methods offer a pathway toward more precise air quality standards that reflect the OP of PM, shifting the regulatory focus from total particle mass to oxidative activity—a parameter more directly associated with health risks.

• Targeted regulatory frameworks: the influence of light exposure and metal–organic interactions on ROS generation suggests the need for region-specific regulations, particularly in areas with high levels of industrial emissions or intense sunlight. Regulations could prioritize control measures for key ROS-generating components, especially transition metals and organic compounds known to drive ROS production in sunlight.

• Enhanced risk assessments: by integrating oxidative potential into public health risk assessment frameworks, regulatory agencies can better understand and address the nuanced health risks associated with PM exposure. This is especially relevant for vulnerable populations such as children, the elderly, and individuals with pre-existing health conditions, who are more susceptible to ROS-induced oxidative stress.

• Research-driven policy development: expanding our understanding of ROS sources and measurement techniques will enable more data-informed policy decisions. For instance, enforcing stricter emission standards in high-risk areas and promoting cleaner technologies could reduce public health burdens linked to oxidative stress and respiratory diseases.

Incorporating oxidative potential into air quality policies, alongside traditional pollutant metrics, would enable more effective public health strategies and regulatory frameworks that address the complex chemistry behind PM toxicity. This integration represents a progressive step toward policies that mitigate the multifaceted impacts of air pollution on public health.

Data availability

No new data were generated or analyzed in this study. The review synthesizes information from previously published studies, as cited in the text.

Author contributions

The contribution of the authors is summarized as follows: conceptualization, C. Y. and V. W. P. D.; resources, Y. Z. and F. C.; data curation, C. Y.; writing—original draft preparation, C.

Y. and V. W. P. D.; supervision, C. Y., F. C., S. D., and Y. Z.; writing—review, V. W. P. D.; editing, G. B., T. L., F. D., and Z. L.

Conflicts of interest

The authors declare no conflict of interest.

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