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Review

Analytical Methods for Oxalate Quantification: The Ubiquitous Organic Anion

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Abstract: Oxalate is a divalent organic anion that has implications across many biological and commercial processes. It is derived from plant sources, such as spinach, rhubarb, tea, cacao, nuts, and beans, and therefore is commonly found in raw or processed food products. Oxalate can also be made endogenously by humans and other mammals as a byproduct of hepatic enzymatic reactions. It is theorized that oxalate is useful for plants to store calcium and protect against herbivory. Clinically, oxalate is best known to be a major component of kidney stones, which commonly contain calcium oxalate crystals. Oxalate can induce an inflammatory response that decreases the immune system's ability to remove renal crystals. When formulated with platinum as oxaliplatin (an anticancer drug), oxalate has been proposed to cause neurotoxicity and nerve pain. There are many sectors of industry that are hampered by oxalate, and some that depend on it. For example, calcium oxalate is troublesome in the pulp industry and the alumina industry as it deposits on machinery. On the other hand, oxalate is a common active component of rust removal and cleaning products. Due to its ubiquity, there is interest in developing efficient methods to quantify oxalate. Over the past four decades several diverse methods have been reported. These approaches include electrode-based detection, liquid chromatography or gas chromatography coupled with mass spectrometry, enzymatic degradation of oxalate with oxalate oxidase and detection of hydrogen peroxide as a product, and indicator displacement-based methods employing fluorescent or UV light-absorbing compounds. It has become clear that no single method will work best for every purpose. This review describes the strengths and limitations of each method, and may serve as a reference for investigators to decide which approach is most suitable for their work.

Keywords: oxalate; oxalic acid

1. Introduction

Oxalate is a divalent organic anion (Fig. 1) that plays a role in a wide variety of fields. It can be derived from plant sources, formed as a byproduct in some physiological reactions, and is even formulated with some drugs currently on the international market. It is best known as a major component of kidney stones (nephrolithiasis), but also plays a role in oxidative stress and inflammation in humans. It is also an important compound in plant physiology and defense [1]. Due to its diverse roles and impact on society, there has been great interest in quantifying oxalate for several decades. The discussion that follows provides more detail on the various fields where oxalate is pertinent, and thereafter we describe the analytical methods used for quantifying oxalate.

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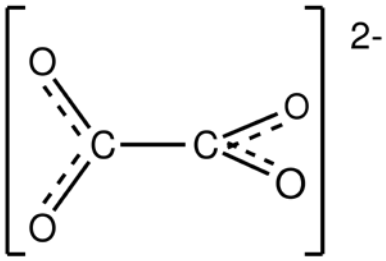

Figure 1. Structure of the oxalate ion.

1.1. Oxalate in Clinical Science

The incidence of nephrolithiasis in the United States is on the rise and is currently estimated to be similar to the prevalence of diabetes. Incidence of nephrolithiasis is 10.6% (men) and 7.1% (women) [2] whereas the prevalence of diabetes is 10.5% [3]. Calcium oxalate is the cause of over 70-80% of patients presenting with renal stones [2,4]. Oxalate is found in human blood at levels typically around 1-5 μM; however, the oxalate level in urine is typically 100 times higher than in blood. Supersaturation of renal ultrafiltrate and urine with oxalate is crucial for formation of renal stones, which usually attach to subepithelial plaques (Randall's plaques) or apatite deposits in the collecting ducts. Oxalate is passively absorbed in the human gut and also absorbed via carrier-mediated mechanisms [5]. Besides the diet, oxalate is formed in humans by the liver as an end product in glyoxylate metabolism by hepatic peroxisomes. Dysfunction of the glyoxylate metabolic pathway is the cause of primary hyperoxaluria type 1, which is a rare autosomal recessive disorder [6,7]. Dysfunction of SLC26A6 (also known as PAT1), a secretory intestinal transporter, has been correlated with increased urinary excretion in mice. SLC26A6 dysfunction has also been shown to cause a net increase in oxalate absorption [8]. Increased blood oxalate causes an increased renal burden and urinary excretion. Studies show that patients who have undergone malabsorptive bariatric surgery, especially Roux-en-Y gastric bypass, approximately double their risk of nephrolithiasis [9]. A link between oxalate and the renin-angiotensin system pathway has also been found showing that angiotensin II can increase intestinal secretion of oxalate, although renal insufficiency may be necessary to induce this interaction [5,10].

1.2. Inflammation/Immunology

When renal epithelial cells are exposed to oxalate, it can incite an inflammatory response via an increase in reactive oxygen species as well as a decrease in glutathione levels. Oxalate exposure has also been shown to impair DNA synthesis and cause cell death. Oxalate impairs monocytes' ability to differentiate into macrophages. Macrophages are important for renal crystal removal. Increased dietary intake of oxalate has been shown to alter immune responses in healthy patients [2].

1.3. Drugs and nutrients containing oxalate or metabolized to oxalate (oxaliplatin, vitamin c)

Oxaliplatin is a platinum-containing anticancer medication (Fig. 2) commonly used to treat a variety of cancers including breast, colorectal, and lung. Once oxaliplatin is metabolized by the body, oxalate ions are one of two major metabolites that are formed. These oxalate ions are linked to negative side effects of the drug including severe nerve pain characterized by allodynia (hypersensitivity to painful stimuli) [11]. Oxalate is hypothesized to cause this neurotoxicity through 2 mechanisms; chelation of calcium by oxalate affects calcium-sensitive voltage-gated sodium channels, and an indirect action on

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voltage-gated sodium channels where oxalate alters intracellular calcium-dependent regulatory mechanisms [12]. Furthermore, oxalate has also been proven to decrease the maximum sodium current peak similarly to the decrease seen from oxaliplatin.

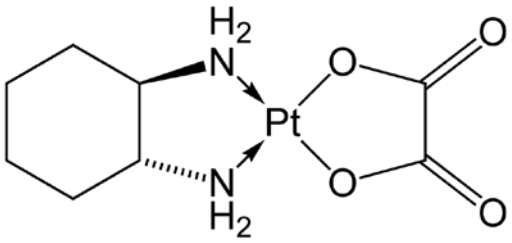

Figure 2. Structure of oxaliplatin.

Ascorbic acid (vitamin C) is an essential component of the human diet as it cannot be synthesized endogenously. It plays important roles in a number of physiological functions including the synthesis of neurotransmitters and collagen, protein metabolism, immune function, nonheme iron absorption, and is also an antioxidant [13]. The breakdown of ascorbic acid by the body results in oxalate formation. Although additional studies are warranted, there have been multiple reports of ascorbic acid increasing a person's risk of kidney stones [14]. One study from Sweden showed that supplemental ascorbic acid (around 1000 mg) intake doubled the risk of kidney stones [15].

1.4. Oxalate in Food Science

Calcium oxalate crystals are found in all taxonomic levels of photosynthetic organisms. Oxalate-rich foods are leafy vegetables like spinach, rhubarb, and tea [6]. Chocolate derived from tropical cacao trees, strawberries, nuts, wheat bran, sweet potatoes, black olives, and most dry beans are also high in oxalate [5,16]. There have been problems in the reporting of oxalate content of foods due to problems with the accuracy of the analytical methods used. Of note, variability in oxalate between food samples also contributes to the challenge of accurately measuring oxalate. For example, changes in growth conditions, plant variety, and the developmental stage of the plant are known to affect oxalate content [16]. Due to this variability, achieving a broad linear range for quantifying oxalate is essential.

1.5. Relevance of oxalate in plants/fungi

Although the role of oxalate in plants has not been completely elucidated, it has been suggested that oxalate is involved in seed germination, calcium storage, detoxification, structural strength, and insect repellant [16]. Calcium oxalate is also important in plants to protect against herbivory and phytotoxins [17,18].

1.6. Oxalate in Industrial Processes

Oxalate is found in, or produced from, some household and industrial products/processes. For example, ethylene glycol is a chemical commonly used in antifreeze and coolant. There have been multiple case reports confirming that ethylene glycol toxicity causes hyperoxalemia, hyperoxaluria, and calcium oxalate crystal formation in the urine because oxalate is a metabolic product of ethylene glycol [19]. SerVaas Laboratories Inc. reports that the core ingredient in Bar Keepers Friend, a common cleaning product, is oxalic acid [20]. In alumina refineries, where bauxite ore is refined to alumina (Al_2O_3), sodium oxalate

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is a major impurity that coprecipitates with aluminum hydroxide. This sodium oxalate is then removed to both remove the impurity from the constantly recycled processing liquor and also to recover sodium [21]. Calcium oxalate is troublesome in the pulp (paper) industry. Oxalic acid is found naturally in wood and as it goes through the bleaching process it deposits on machinery, decreasing product quality and profitability [22].

2. Methods for Detection and Quantification of Oxalate

Several diverse methodologies for detecting and quantifying oxalate have been reported over the past 4 decades. These methods are introduced here, and then more detailed descriptions with references follow. A tabular summary, including limits of detection and quantification, is provided (Table 1).

Table 1 - Methods used for detection and quantification of oxalate. LOD, limit of detection; LOQ, limit of quantification.

Method Summary	LOD µM	LOD µg/mL	LOQ µM	Additional Characteristics	Strengths	Limitations	Ref.	Year Published
Enzymatic analysis by flow injection analysis	34.0	2.99	NR, but linearity was shown up to 500 µM	Flow injection analysis (FIA) was used to measure oxalate in urine. A column reactor of immobilized oxalate oxidase was used. Generated H2O2 was measured by chemiluminescence via a reaction of luminol with hexacyanoferrate(III). L-ascorbic acid interference with the assay was unable to be eliminated with the use of sodium nitrite.	Highly specific for oxalate.	Unable to remove L-ascorbic acid as an interfering substance due to spontaneous conversion to oxalate	[23]	1994
Enzymatic analysis with HPLC	1.5	0.13	NR, but linearity was shown up to 700 µM	An anion exchange column was used prior to reaction of oxalate with barley oxalate oxidase in the flow path. Product, H2O2 was measured with an amperometric detector.	Oxalate was resolved in 8.2 min, with comparable accuracy to commercial enzyme-based kits. Higher sensitivity than enzyme-based kits.	Requirement for immobilization of oxalate oxidase.	[24]	1997
Alkylamine glass bound oxalate oxidase measured by a color reagent	10.2	0.9	NR, but linearity was shown up to 300 µM	Urinary oxalate was measured with an alkylamine glass-bound oxalate oxidase purified from leaves of Amaranthus spinosus. H2O2 is generated from urinary oxalate, which is measured with a colored reagent containing 4-aminophenazone, phenol, and horseradish peroxidase. The chromogen formed is read at 520 nm. Recovery of oxalate was 98%. Results were comparable to the Sigma kit method (r=0.98)	Oxalate-oxidase beads were reusable up to at least 300 times.	48-h reaction time required. Use of hazardous reagents (glutaraldehyde and phenol)	[25]	1999

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Catalase model compound with oxalate oxidase quantified by UV-vis	2.0	0.176	2.0	Urinary oxalate was determined based on a catalase model compound MnL(H2O)2(ClO4)2 (L = bis(2-pyridylmethyl)amino)propionic acid). Oxalate is decomposed with oxalate oxidase into CO2 and H2O2. H2O2 is converted to H2O and O2 by the catalase model compound, forming a color compound. Absorbance of the formed compound is measured. Linear from 0.002-20 mM oxalate. Results are consistent with enzymatic and HPLC methods. The compound modeled off catalase avoids the need for enzymes.	High sensitivity	Real urine samples yielded a higher oxalate concentration than expected	[26]	2009
Oxalate oxidase-based biosensor	20.0	1.76	NR, but linearity was shown up to 5 mM	Oxalate oxidase was used with SIRE (sensors based on injection of the recognition element) technology. Analysis was selective, simple, and inexpensive. Linear between 0-5 mM or more with a shorter reaction time. Results were comparable to a colorimetric method.	Minimal enzyme degradation and correction for matrix-related interferences. Rapid analysis time (2-9 min)	Limit of detection relatively high.	[27]	2003
Amperometric biosensor used on urine	11.4	1.0	34.1	Oxalate oxidase enzyme was immobilized on a magnetic solid containing an electrode modified with Fe (III)-tris-(2-thiopyridone) borate. Oxalate quantification was linear between 3.0-50.0 mg*L-1.	No sample treatment, small volume, and simple instrumentation.	Relatively high limit of quantification.	[28]	2012
LC-MS/MS and anion exchange chromatography	3.0	0.264	100.0	Urinary oxalate was quantified using weak anion exchange chromatography and MS/MS. Used a Waters OASIS WAX column, and detected oxalate 88.6>60.5 m/z transition and a 90.5>61.5 transition for 13C2-oxalate. Electrospray negative ion mode was used. Oxalate eluted at 1.2 min, 95% recovery, LOQ was 100 umol/L. Linear to 2212 umol/L. Oxalate eluted away from area of ion suppression. No interference from organic acids. Quantitative results were comparable to enzyme assay	Retention time afforded resolution from areas of ion suppression. Short (1.2 min) retention time.	Relatively high limit of quantification.	[29]	2006
LC-MS/MS and WAX Solid Phase Extraction (SPE)	NR	NR	60.0	Measured urine citrate and oxalate after a simple weak anion exchange solid phase extraction (WAX-SPE) clean-up procedure. Detected with electrospray-negative ion mode. Linear from 60 to 1388 umol/L	Reaction monitoring of oxalate 88.9>60.85 allows for more confident identification. Oxalate eluted at 0.29 min. 100% oxalate recovery.	Manual SPE plate loading, eluent evaporation, and reconstitution. Required ^{13}C use as an internal standard to correct for matrix-derived ion suppression.	[30]	2018
GC-MS/MS	NR			Stable isotopes (^{13}C) of oxalate and glycolate were continuously infused into human subjects to monitor tracer-to-tracee ratios (hepatic metabolism). Plasma samples were derivatized with MTBSTFA and analyzed by GC-MS/MS.	Ideal for determining efficacy of therapeutic interventions for treating primary hyperoxaluria (hepatic oxalate synthesis).	Not designed for quantifying absolute oxalate concentrations.	[33]	2020

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GC-MS	NR, but 25 nmol mass was reported as detectable			Used a stable isotope (^{13}C) of oxalate as an internal standard added to urine. Oxalate was converted to its isopropyl ester using propaone-2-ol-HCl,	N/A	Requires a 3-h precipitation step and drying step. Uses chloroform	[31]	1987
Capillary GC-flame ionization vs. GC-MS	NR, but oxalate was quantified as low as 1.5 µM			Plasma oxalate was extracted and derivatized with MTBSTFA prior to capillary gas chromatography and flame ionization. GC-MS showed a 0.938 correlation coefficient with the method, wherein ^{13}C-oxalate was used as an internal standard.	Reported quantifying oxalate as low as 1.5 µM	24-h derivatization step, and a 57.9% oxalate recovery when extracted with ethyl acetate.	[32]	1988
Anion exchange column chromatography and conductivity detection	NR	NR	5.68 x 10^{-6}	Anion exchange chromatography with conductivity detection was shown to be superior to capillary electrophoresis for estimating low oxalate concentrations.	Very high sensitivity. Complete oxalate recovery. Linear response.	Retention time of oxalate was up to 15.2 min. Column "poisoning" was reported, wherein food components altered column chemistry over time. Oxalate appeared to elute on a broader peak (not baseline resolved) in apple extract. However, this was not observed in foods of higher oxalate content.	[16]	2000
Oxalate-selective membrane electrode	0.05	4.4 x 10^{-3}	0.05	Used 2,2′-[1,4-butandiyle bis(nitrilo propylidine)]bis-1-naphtholato copper(II). Linear detection of oxalate from 0.05 to 100,000 uM	Sensitive. Fast response time (10-15 s) and could be used for more than 3 months.	Only used in water samples	[34]	2006
Microchip electrophoresis	0.180 , down to 0.019 with field-amplified sample stacking.	1.67 x 10^{-3}	0.04	Used a zwitterionic surfactant with affinity for solvated anions (N-tetradecyl,N,N-dimethyl-3-ammonio-1-propanesulfonate). Separation was performed at pH 4.7, permitting pH manipulation of oxalate's mobility.	Sensitive. 1-min separation. Allows continuous online monitoring of aerosol composition.	Tested with atmospheric aerosols, but not biological samples. Poor resolution of oxalate and the internal standard at higher concentrations. Loss of linearity above 300 µM oxalate. Change in bulk solution conductivity with higher analyte concentrations, requiring internal standard-based correction.	[35]	2009

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Capillary electrophoresis coupled with 254 nm UV absorbance	NR	NR	5.68 x 10^{-6}	Various foods were processed and heated to extract oxalate. Oxalate was resolved using capillary electrophoresis, and quantified by measuring change in UV absorbance of oxalate-displaced chromate.	Very high sensitivity. Complete oxalate recovery. Linear response.	Less specific compared to mass spectrometry or enzyme-based methods. Electrolyte concentration required adjustment for oxalate concentration. Sample heating for 1 h required.	[16]	2000
Oxalate inhibition of reaction of curcumin nanoparticles with Fe(III) -- colorimetric	0.87	0.077	1.70	Oxalate inhibited the reaction of curcumin nanoparticles (CURN) with Fe (III) in acidic media. Oxalate was measured by absorbance of CURN with Fe (III) at 427 nm. Absorbance intensity was linear with oxalate from 0.15 to 1.70 ug/mL. The method was used for detection of oxalate in water, food, and urine.	Sensitive and simple. Effective for water, food, and urine samples.	Interference from copper and carbonate/bicarbonate. Unknown if there is interference from other substances.	[37]	2018

Enzyme-based methods employing oxalate oxidase have realized a niche in commercial oxalate detection kits due to their simplicity and relatively common instrumentation for reading samples. There appears to be promise in conjugating oxalate-degrading enzymes with solid supports, as they can be washed and reused. From a commercialization perspective it may be useful to develop a device with oxalate-oxidase coupled to beads or a monolithic solid in order to circumvent some sample preparation steps associated with enzyme dilution. Alternatively, success has been achieved using the sensors based on injection of the recognition element (SIRE) approach, wherein small quantities of oxalate oxidase are added for one-time use.

Liquid chromatography-mass spectrometry (LC-MS) may not become a method of choice for oxalate due to its low sensitivity compared to other methods, and also compared to other analytes using LC-MS. Perhaps the primary advantage is more confident identification of oxalate using MS/MS, which may be acceptable if oxalate content is above the limit of quantification. GC-MS has also not realized much utility in oxalate detection, possibly due to the time required for derivatization. However, it is clear that investigators continue to explore GC-MS methods for oxalate detection.

The most sensitive oxalate detection methods to date are electrode based. These approaches do, however, often require corrections based on bulk solution conductivity changes, and loss of linearity at higher oxalate concentrations. In addition, the separation columns/capillaries coupled with electrode-based detection are often susceptible to fouling when real food or biological samples are used.

Finally, indicator displacement methods wherein oxalate turns on or turns off fluorescence, or alters UV absorbance, can be rapid and instrumentally simple, but are limited by lack of specificity of the indicator.

Therefore, to determine which methodology is most suitable for quantifying oxalate one must answer several questions first: What sensitivity is needed? What linear range is needed? How complicated is the sample matrix and what is the likelihood of interfering compounds? What instrumentation is available? What expertise is needed to employ the method? It is clear that no single method will be suitable for every investigation. A summary of the different approaches is presented in the following sections.

2.1. Enzyme-Based Methods

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Enzyme-based detection of oxalate has been employed for several decades, and is used in several commercially available kits. In 1994, oxalate oxidase was immobilized in a column, where it was used to metabolize urine-borne oxalate into hydrogen peroxide and carbon dioxide [23]. In this flow injection analysis method, chemiluminescence was measured from the reaction of hydrogen peroxide with luminol and hexacyanoferrate (III). The limit of detection was 34 μM (2.99 μg/mL), and linearity was confirmed up to 500 μM. The advantage of this method is that oxalate oxidase is specific for oxalate as a substrate; however, the disadvantage is that L-ascorbic acid was unable to be removed as an interfering substance due to its spontaneous conversion to oxalate.

In a related method in 1997 HPLC was employed with an anion exchange column to resolve urinary oxalate prior to reaction with immobilized oxalate oxidase isolated from barley [24]. The product, H_2O_2 was quantified using an amperometric detector. Oxalate eluted at 8.2 min, with values comparable to that of a commercial enzyme-based kit for several different concentrations. The limit of detection was 1.5 μM, which is more sensitive than the enzyme kits currently on the market (~20 μM reported). Linearity was reported up to at least 700 μM oxalate.

In 1999, urinary oxalate was measured with an alkylamine glass-bound oxalate oxidase purified from leaves of *Amaranthus spinosus* [25]. The hydrogen peroxide generated in the reaction was measured using 4-aminophenazone, phenol, and horseradish peroxidase. This reaction yielded a chromogen that absorbed 520 nm light. The results of this protocol were reportedly comparable to that of the commercially available kits. The limit of detection was 10.2 μM (0.9 μg/mL) and linearity was shown up to 300 μM. Limitations of this method, when first published, are likely mitigated by improvements in biotechnology related to genetic engineering and protein purification. Some potential limitations are the use of glutaraldehyde in the immobilization process, and phenol in the detection step, which must be handled with some precautions, in addition to the 48-h reaction time for coupling oxalate oxidase to the beads. A major strength of the method is that the oxalate-oxidase-bound beads were reusable for at least 300 times after simple washing and storage in distilled water. If combined with modern technology it appears feasible to construct a device, based on this method, for direct-to-consumer use.

In the following year, urinary oxalate was again detected after reaction with oxalate oxidase; however, a synthetic compound, $MnL(H_2O)_2(ClO_4)_2$ (L = bis(2-pyridylmethyl)amino propionic acid), modeled off the catalase enzyme was thereafter reacted with hydrogen peroxide that had been generated from oxalate oxidase, forming a chromogen [26]. When absorbance of the chromogen was measured at 400 nm the limit of detection for oxalate was found to be 2.0 μM (0.176 μg/mL). The linear range was 2.0 μM to 20 mM. It was also observed that adjusting the concentration of the catalase mimetic had a significant influence on the assay sensitivity. For example, 6 mM was required to detect the lower oxalate concentrations, whereas 1 mM was adequate for detecting 20 mM oxalate. A reported limitation of the method was that in real urine samples the calculated oxalate concentration was exceedingly higher than what is typically found. The investigators concluded that an additional substance(s) in urine may have reacted with the catalase mimetic to increase absorbance at 400 nm. The identity of the interfering substance(s) was not determined. Although this limitation precludes clinical use of the assay for oxalate quantification, revealing the limitation should lead to further exploration to determine the scope of reactivity of the catalase mimetic.

In 2003, oxalate oxidase was used with SIRE (sensors based on injection of the recognition element) technology to measure oxalate in urine [27]. In this method, oxalate oxidase was introduced into the reaction chamber by flow-injection, where it was free to react with oxalate from a sample. The hydrogen peroxide generated from the reaction was oxidized at the electrode, where the signal was then received. The advantage of this method is that oxalate oxidase is not immobilized to a support, but rather, it is used once in a small quantity before being discarded. This approach ensures that the enzyme is not degraded from thermal or other factors that can be troublesome for repeat-use biosensors.

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Moreover, the SIRE method allows the sample response to be easily measured in absence of enzyme, to correct for matrix-related interferences. The analysis time for this approach was 2-9 min, with high selectivity imparted by oxalate oxidase, and a limit of detection of 20 μM (1.76 μg/mL). Linearity was shown up to 5 mM.

In 2012 a group of investigators reported coupling oxalate oxidase-mediated oxalate metabolism with amperometric flow detection for quantifying oxalate in human urine [28]. In the study, oxalate oxidase was immobilized on a magnetic solid that was transiently retained on an electrode modified with Fe (III)-tris-(2-thiopyridone) borate. This complex served as a mediator that circumvented known issues with direct amperometric measurement of H_2O_2, such as interference from ascorbic acid and uric acid in urine. The reaction scheme is represented in Fig. 3. The limit of detection was 11.4 μM (1.0 μg/mL) with a limit of quantification of 34.1μM. The advantages of this method are no need for sample treatment, small sample volumes, and relatively simple equipment.

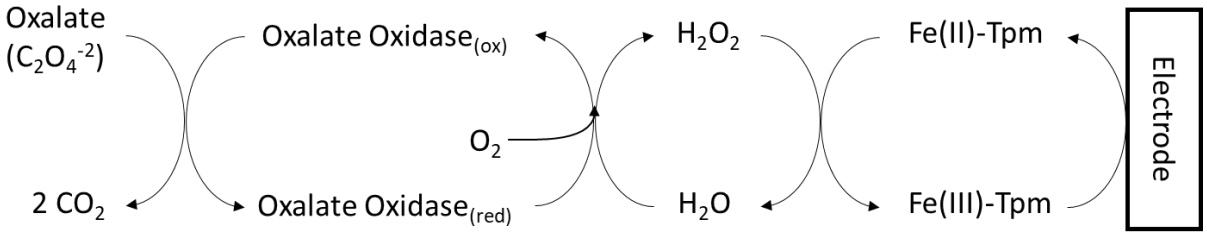

Figure 3. Coupling of immobilized oxalate oxidase with amperometric detection. Oxalate oxidase was immobilized on magnetic solids, which were transiently retained on an electrode. Fe(III)-tris-(2-thiopyridone) borate (Fe(III)-Tpm) was used as a mediator between enzymatically generated hydrogen peroxide and the electrode.

2.2. Liquid Chromatography-Mass Spectrometry-Based Methods

Liquid chromatography coupled with mass spectrometry (LC-MS or LC-MS/MS) has become the method of choice for many analytes in food and drug analysis. High sensitivity, and unparalleled selectivity, is generally a major advantage of MS; however, the limit of quantification of oxalate using electrospray ionization with MS has not realized the sensitivity advantage. For example, in 2006 urinary oxalate was quantified using a weak anion exchange chromatography column and tandem mass spectrometry with electrospray ionization in the negative ion mode [29]. The limit of detection was 3.0 μM (0.264 μg/mL), and the limit of quantification was 100 μM. Linearity was demonstrated up to 2.2 mM. This limit of quantification is higher than that of some commercially available enzyme-based kits. However, one advantage of the method was a short (1.2 min) retention time with adequate resolution from areas of ion suppression.

In 2018 a similar method was used, wherein a solid-phase extraction plate with weak anion exchange activity was employed in resolving urine oxalate [30]. When electrospray ionization in the negative ion mode was used with tandem mass spectrometry a limit of quantification of 60 μM was found. Linearity was demonstrated up to 1.39 mM. Both this method and the former used $^{13}C_2$ oxalate as an internal standard to correct for matrix-derived ion suppression. One advantage of the method is the ability to simultaneously quantify citrate, which may be useful due to its ability to sequester calcium and thus reduce the risk of calcium oxalate stones. Some limitations of the method include a lower sensitivity than other methods, and the time required for solid phase extraction steps, including a drying phase, and several wash phases. These disadvantages can likely be circumvented with instrumental automation.

2.3. Gas Chromatography-Based Methods

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A GC-MS method was also reported in 1987 for determining urinary oxalate with ^{13}C-oxalate as an internal standard [31]. This protocol required a 3-hour precipitation step for oxalate with calcium, derivatization to its isopropyl ester with propane-2-ol-HCl, and the limits of detection and quantification were not directly assessed. However, linearity was shown from 25 µL to 100 µL of 1.0 mM oxalate after drying, derivatization, and chloroform extraction.

In 1988, plasma oxalate was measured by capillary GC with flame ionization detection, and compared with GC-MS, using ^{13}C-oxalate as an internal standard in the latter method [32]. In this protocol oxalate was extracted with ethyl acetate and derivatized with MTBSTFA for 24 h. A limitation of the method, other than the reaction time, was a 57.9% oxalate recovery. Although a limit of detection was not reported, oxalate was quantified as low as 1.5 µM in plasma. This reported sensitivity is not typically achieved with the majority of other methods discussed in this review. Lastly, the correlation coefficient between flame ionization detection and mass spectrometry was 0.938.

A recent report (2020) demonstrated that gas chromatography coupled with tandem mass spectrometry could be instrumental in measuring the oxalate synthesis rate in human subjects with or without primary hyperoxaluria (liver-generated oxalate) [33]. In this method ^{13}C-labeled oxalate and glycolate were continuously infused into human subjects to monitor tracer-to-tracee ratios (TTRs). Plasma samples were derivatized with MTBSTFA prior to GC-MS/MS analysis. The proposed major utility of the method is in measuring the efficacy of therapeutic interventions for preventing oxalate synthesis. The method was not designed to measure absolute oxalate concentrations, but rather tracer/tracee ratios. Therefore, limit of detection and quantification were not reported. However, the TTR calibration curve was linear with an R^2 of 0.9999.

2.4. Electrode-Based Non-Enzymatic Methods

In 2000, the oxalate content of various foods was quantified using anion exchange chromatography coupled with conductivity measurement [16]. This method was compared with capillary electrophoresis (discussed in the next section). The anion exchange method exhibited very high sensitivity (limit of quantification of 5.68 pM) compared to other reported methods. The response was linear and oxalate recovery was complete when mechanically processed food was heated with acid. A limitation was column "poisoning", a term used to describe contamination of the column with food substances and diminished resolution with repeated use.

In 2006 an oxalate-selective membrane electrode based on polyvinyl chloride and the complex and ionophore, 2,2′-[1,4-butandiyle bis(nitrilo propylidine)]bis-1-naphtholato copper(II) (Fig. 4), was used to quantify oxalate in water [34]. The limit of detection was 0.05 µM (4.4 x 10^{-3} µg/mL), and the linear range was 0.05 µM to 1.0 x 10^5 µM. Noted advantages, other than the sensitivity and dynamic range, were the fast response time of 10-15 seconds, and the ability to use the same electrode for 3 months. In addition, the electrode was several orders of magnitude more selective for oxalate than other anions, with the exception of perchlorate and OH^- (when tested in alkaline conditions). However, the utility of the method in a more complex sample matrix, such as urine or plasma, is unknown. If refined to maximize selectivity and proven to be suitable in complex fluids this method could potentially be preferred due to its minimal need for sample handling or complex equipment.

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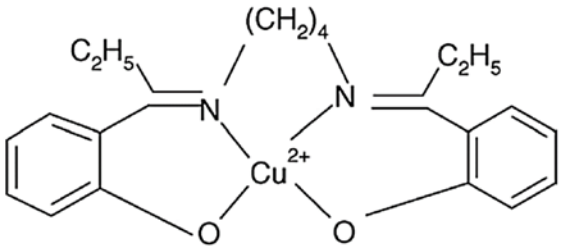

Figure 4. Ionophore used in [33]. The ionophore, 2,2-[1,4-butandiyle bis(nitrilo propylidine)]bis-1-naphtholato copper(II) was used in a PVC-based ion-selective electrode for detection of oxalate.

In 2009 a method for sensitive and selective detection of oxalate in aerosols using microchip electrophoresis was reported [35]. In this study the investigators used micellar electrokinetic chromatography (MEKC), which takes advantage of the ability of surfactant micelles to modify separations of weakly solvated anions. Using this approach, oxalate, being a highly-solvated anion, was readily separated from the micelle-influenced analytes. In addition, picolinic acid was used in order to chelate metals that would otherwise bind and reduce the detector response to oxalate. Detection was achieved with a Dionex conductivity detector, and the limit of detection for oxalate was 180 nM (1.67×10^{-3} μg/mL), with a 19 nM limit when field-amplified sample stacking was used. This sensitivity is a major advantage of the method, exceeding that of a large majority of other methods reported to date. In addition, a major advantage of this method is the ability for continuous online monitoring of aerosol composition due to the sample run times of less than 1 minute. Disadvantages of this method include a loss of response linearity above 300 μM, poor resolution between oxalate and the internal standard at higher concentrations, and a change in bulk solution conductivity with higher analyte concentrations. The latter limitation needs to be corrected by internal standard addition. Finally, this method has not been tested in biological samples, such as urine or plasma.

2.5. Indicator Displacement-Based UV, Colorimetric, and Fluorescence Methods

There is substantial interest in developing indicator-displacement-based methods for detecting oxalate that do not require enzymes, liquid chromatography, or mass spectrometry. Many of these methods quantify fluorescence quenching, or, more often, gain of fluorescence upon addition of the analyte [36]. The principle of operation is shown in Fig. 5. A capillary electrophoresis method coupled with indicator displacement and UV absorbance [16] and a colorimetric method [37] have also been reported. These approaches are briefly discussed here.

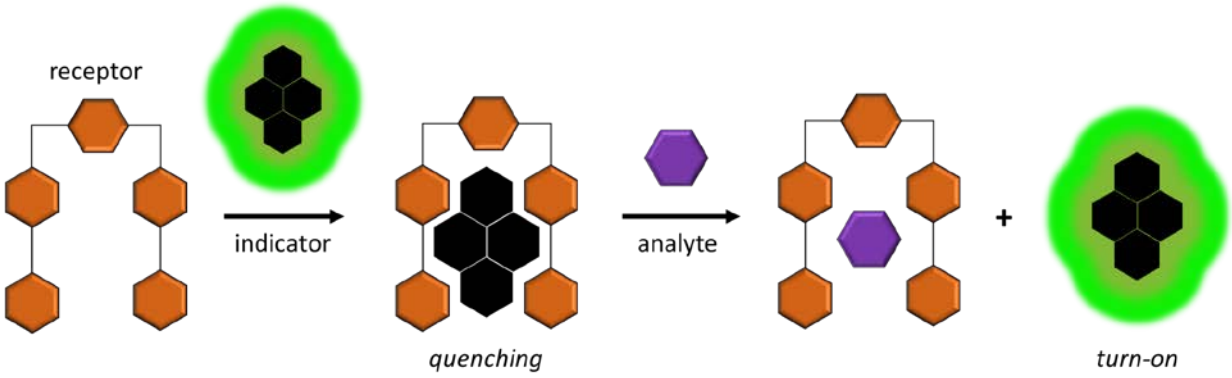

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Figure 5. Principle of operation of indicator displacement-based fluorescent chemosensors. The receptor (e.g., macrocyclic metal complex) sequesters the indicator, inhibiting fluorescence. The analyte of interest is added to displace the indicator, regaining fluorescence.

In 2000, the oxalate content of foods was estimated via capillary electrophoresis coupled with the UV absorbance (254 nm) response to oxalate-mediated chromate displacement [16]. The reported limit of quantification was 5.68 pM, which is the greatest sensitivity that we have found reported to date. Limitations included the need for adjustment of electrolyte concentration for samples with low oxalate concentrations, and heating samples for 1 h. There is also potential for oxalate to co-elute with interfering compounds, but this limitation was not observed in the study.

In 2018 a colorimetric method was developed wherein oxalate inhibited the reaction of curcumin nanoparticles with Fe (III) in acidic media [37]. Absorbance of 427 nm light by the nanoparticles showed a linear increase proportional to oxalate concentration from 0.15 to 1.70 μg/mL. The limit of detection was 0.87 μM (0.077 μg/mL). The method was demonstrated to work well for quantification of oxalate in water, food, and urine samples. Advantages of the method include simplicity and sensitivity. A potential limitation is interference from other substances, which was demonstrated for copper and carbonate/bicarbonate, and was not assessed for other common anions in urine, such as citrate and urate. Indeed, all indicator displacement methods are limited by either known interferences, or untested putative interferences.

A recent report by Hontz et al. revisited a fluorescence-based indicator displacement method, clearly demonstrating that interference from untested compounds in biological samples may be more prevalent than is sometimes suggested [36]. Specifically, it was shown that a dinuclear copper(II)-based macrocycle with the ability to quench eosin Y fluorescence, although reported as oxalate selective, exhibited a stronger response to citrate and oxaloacetate. Other copper-based oxalate sensors, also based on reversing quenching, have been reported [38,39]. The reported sensitivities of these methods are in the low μM range; however, we have found that all studies to date have not performed a comprehensive assessment of potentially interfering compounds. For example, Rhaman et al. reported a nickel-based macrocycle that bound to oxalate in water [40]. Oxalate binding to the macrocycle triggered a red shift in eosin Y fluorescence with a corresponding visible color change. Potential interfering ions tested in this study included F^-, Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, SO_4^{2-}, and PO_4^{3-}. Although none of the ions demonstrated interference, organic anions (e.g., citrate, urate, or oxaloacetate), were not tested. In contrast to reversing fluorescence quenching, at least one investigation reported reversal of a fluorescence "turn on" response [41]. Specifically, Tang et al. showed that Zn^{2+} could turn on fluorescence of a binaphthol-quinolone Schiff base. This gain in fluorescence could then be reversed via oxalate-mediated chelation and displacement of the zinc ion. In this study, phthalate, isophthalate, terephthalate, succinate, glutarate, adipate, and malonate showed no significant interference with the assay when tested at an equimolar concentration to oxalate; however, other anions were not tested, and it remains unknown whether high concentrations of the tested anions would interfere.

Conflicts of Interest

No conflicts of interest exist

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