

Review

Detection and determination of nitrate and nitrite: a review

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Abstract

A review of the strategies employed to facilitate the detection, determination and monitoring of nitrate and/or nitrite is presented. A concise survey of the literature covering 180 reports submitted over the past decade has been compiled and the relevant analytical parameters (methodology, matrix, detection limits, range, etc.) tabulated. The various advantages/disadvantages and limitations of the various techniques have been exposed such that the applicability of a technique developed for one type of matrix can be meaningfully assessed before attempting to transfer the technology to another. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Nitrate and nitrite are ubiquitous within environmental, food, industrial and physiological systems, and while our understanding of their role within such matrices has increased, a substantial degree of uncertainty and speculation remains. These ions have been profitably exploited throughout the ages to further the development of our various societies but there is no doubt that our affection for them has waned in recent years. Our incessant use of, and indeed reliance upon,

these versatile agents combined with revelations of their potential toxicity have raised numerous concerns [1,2]. These problems have been widely recognised, and, as a consequence, statutory frameworks aimed at controlling their level within the wider environment and within food products have been imposed in most industrialised countries [3,4]. The restrictions placed upon the commercial utilisation of these ions have eased some of the apprehensions raised by the medical community but the establishment of adequate controls can only be achieved by fully exposing their influence on the various pathways that govern environmental and physiological well being.

Our need and desire to monitor these ions are unquestionable, yet their ubiquity can pose a significant challenge to the analytical community.

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Few techniques possess sufficient generic applicability to enable their detection amongst the huge number of potential interferences that can be encountered within environmental, food, industrial and physiological samples. As such, a large number of protocols, encompassing almost all major analytical methodologies have been developed to overcome the peculiarities of the various matrices. The aim of this review has been to provide a representative survey of the scientific literature covering the detection of these important analytes and provide a concise appraisal of the relative merits of each approach. A number of excellent reviews have been compiled over recent years, but each has narrowed their remit to specific matrices or techniques [5–10]. Through tabulating the various analytical parameters (detection limit, range, matrix, etc.) of each system and exposing their advantages and limitations, it was hoped that the specialism divides inherent within such a diverse subject such could be bridged.

The decision to cover both nitrate and nitrite was made on the basis that their chemistries are inextricably linked, and as such, one is rarely found without the other. Indeed, their inter-conversion, particularly the chemical reduction of nitrate to the more reactive nitrite, features strongly within many of the reports and is often the only way in which the relatively inert nitrate ion can be detected. The simultaneous detection and speciation of these ions has, however, gained increasing interest as the checks and balances that mediate many environmental processes have become clearer. Some of the more common reaction pathways that form the backbone of the detection strategies assessed herein are shown in Fig. 1.

2. Background perspective

Nitrate and nitrite have become intertwined with domestic life, and it is effectively impossible to engage in everyday activities without encountering these ions or the products of their use. The chemical versatility of these agents has ensured their utilisation within a multitude of industrial processes ranging from the manufacture of fireworks to the production of the latest dyes. Their

anti-microbial action has been recognised for centuries and still used for the preservation of meat produce (E249, E250, E251 and E252) today (UK Ministry of Agriculture, Fisheries and Food, <http://www.maff.gov.uk>). Despite the huge number of products that are reliant upon these ions, it is their association within environmental issues that has captured the interest of the public and a significant proportion of the scientific community. Inputs of nitrate and nitrite to the environment can occur through industrial and domestic combustion processes with gaseous NO_x species converted to NO_3^- through photochemical conversion within the atmosphere [11–15]. The vast majority, however, arise from agricultural sources [16].

The misuse of inorganic fertilisers combined with the more general mismanagement of our natural resources has been suggested as resulting in the perturbation of both local and global nitrogen cycles [16,17]. The consequences of our environmental manipulations are as yet uncertain, and therefore monitoring the ecological fate of nitrate and nitrite has gained increasing importance. The high solubility and mobility of these ions within the soil and our continued reliance on inorganic fertiliser has led to reports that “run off” is a continual hazard wherever agricultural processes are in close proximity to surface water [18]. Eutrophication of lakes and more recently coastal

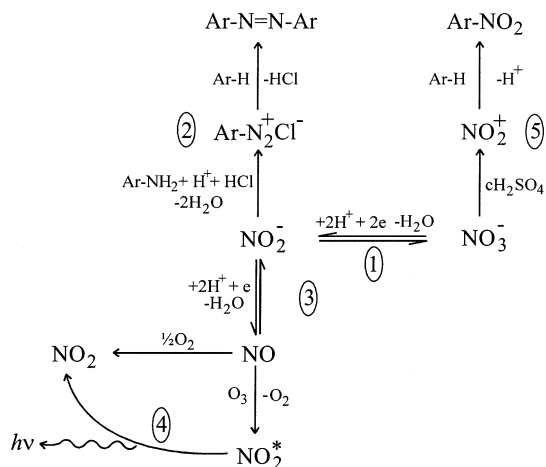


Fig. 1. Common reaction pathways that form the basis of nitrate/nitrite detection strategies.

outfalls are thought to result in the generation of algal blooms that wreak havoc with local ecological systems [19,20]. The contamination of edible shellfish and the occurrence of “red tides” of potentially toxic algae near tourist resorts can also impart a degree of economic misery to the afflicted communities [21].

The potential contamination of groundwater through the percolation of nitrates through natural aquifers presents the most immediate risk to health [1,2] and as such, the maximum permissible level for these ions in drinking water supplies is often levied and currently stands at 50 mg/l in the UK [3,4,22]. The two main threats to health that arise from the ingestion of these ions are reported as “blue baby” syndrome and gastric cancer [1,2,23,24]. In both cases, the principal protagonist is nitrite obtained directly from contaminated water supplies or derived from the reduction of nitrate by the multifarious bacterial colonies that reside within the mouth. Passage of nitrite into the bloodstream results in the irreversible conversion of hemoglobin to methemoglobin with oxygen uptake and transportation compromised [2]. This is particularly hazardous for infants, given their limited physical stature and the susceptibility of their neural development to impeded oxygen transport. A more contentious health concern is the possible formation of carcinogenic nitrosoamines within the acidic conditions of the stomach and their subsequent implication in the pathology of gastric cancer [1,25]. Upon reaching the stomach, nitrite is converted to nitrous acid, which can act as a powerful nitrosating agent. While nitrosoamines can be carcinogenic, conclusive evidence linking nitrite ingestion with the stomach cancer remains elusive [2].

The presence of nitrate and nitrite within physiological systems is often viewed with considerable and, as noted above, justifiable concern. Ingestion is not, however, the sole source by which these ions can arise within physiological systems. The endogenous production of nitrate and nitrite within tissue can occur as a result of the activity of their more transient cousin, nitric oxide [26]. The reaction of nitric oxide with oxygen leads to the production of nitrite [1], and it is through the greater stability of this ion that the action of NO can be detected [27].



Nitric oxide has been shown to play an important role in many metabolic functions, including the regulation of vascular tone, inhibition of platelet aggregation, neurotransmitter, cytotoxic agent, thrombosis, and inflammation, and may also play a role in the immune system [28–30]. The measurement of nitrite can therefore provide a reliable measurement of NO action within the body, and, as such, can be used as a biomarker that enables physicians to gauge the health of an individual [31]. This has particular importance when assessing inflammatory processes as the level of nitrite can be correlated to the degree of injury. Among the more common ailments for which the monitoring of nitrate and nitrite can be beneficial are sepsis [32], infectious gastroenteritis [33], meningitis [34], Parkinson’s disease [25], minimal change nephritic syndrome in children [35], preeclampsia [36] and rheumatoid arthritis [37].

3. Detection methodologies

The techniques that have been marshalled in our attempt to monitor nitrite and nitrate are compiled in Tables 1 and 2, respectively. A third table is included comprising reports that have dealt with the analysis of both analytes either simultaneously or sequentially. Simultaneous techniques include examples such as electrochemical and capillary electrophoresis, whereby the analytes are detected independent of one another in a single measurement. Sequential analysis is formed on the basis of detecting the more versatile nitrite anion initially, followed by bulk reduction of the sample (e.g. Cu/Cd column) to ensure that all the nitrate is converted to nitrite and repeating the nitrite analysis. Calculation of the nitrate concentration can then be obtained by difference. The characteristics of each strategy have been distilled and critically appraised according to the methodology applied and are discussed within the following sections.

It can be seen that there are nearly as many entries for nitrate as there are for nitrite. This observation must be clarified in that while the

Table 1
Parameters for nitrite detection

Technique	Matrix	Detection limit (μM)	Detection range (μM)	RSD%	FIA sample throughput/h	Reference
ED	Saliva/urine/river water	6	4000–80 000	5.0	N/A	[5]
Fluorescence	Aqueous	N/A	0.045–15	3.0	N/A	[54]
Fluorescence	Aqueous	N/A	0.023–15	3.0	N/A	[54]
Greiss	Aqueous	N/A	2–30	3.0	N/A	[54]
Visible	Aqueous	0.098	0.11–540	2.2	N/A	[72]
Visible	Water/food/soil	0.15	0.87–300	N/A	N/A	[73]
Greiss	Water	0.29	0.29–3.5	4.0	15	[77]
Greiss	Water	0.018	0.018–0.43	4.0	3	[77]
Visible	Well/waste water	0.065	0.32–16	3.0	N/A	[78]
Visible	Aqueous	0.20	1–100	2.6	N/A	[79]
Chemiluminescence	Water	N/A	0.25–65	1	N/A	[81]
ED	Water	N/A	0.1–50	N/A	N/A	[81]
Fluorescence	Saliva	0.043	N/A	2.8	40	[82]
Fluorescence	Spiked water	0.16	0.16–8.7	N/A	N/A	[84]
Fluorescence	Water	0.030	0.215–14	3	N/A	[85]
Fluorescence	Tap/lake water	0.002	0.017–2.4	4.9	N/A	[86]
Fluorescence	Aqueous	0.12	0.22–2.6	N/A	N/A	[87]
Fluorescence	Water/food	0.054	0.22–13	N/A	N/A	[88]
Fluorescence	Fish	1.1	5.0–100	N/A	N/A	[89]
Fluorescence	Soil/water	N/A	0–8.7	3	N/A	[90]
Fluorescence	Aqueous	0.059	1.7–28	N/A	N/A	[91]
ED	Egg/water	1	10–100	4.0	N/A	[119]
ED	Saliva	0.005	5–10 000	0.82	N/A	[138]
ED	Saliva	0.0005	5–10 000	0.82	N/A	[138]
ED	Water	0.1	0.2–0.8	1	N/A	[139]
ED	Aqueous	1	5–20 000	3.8	N/A	[140]
ED	Aqueous	1.0	5.0–10 000	10.5	N/A	[141]
ED	Water	N/A	50–30 000	N/A	N/A	[142]
ED	Water	0.03	0.05–500	2.9	N/A	[144]
ED	Aqueous	0.043	21–210	N/A	N/A	[145]
ED	Aqueous	0.25	1–30	0.42	10	[148]
ED	Biological fluids	10	10–10 000	N/A	N/A	[151]
ED	Water	0.13	0.43–13	1.15	N/A	[154]
ED	Water	0.01	500–5000	N/A	N/A	[156]
ED	Aqueous	2.2	5.0–10 000	N/A	N/A	[162]

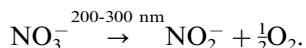
majority of the detection strategies for nitrate actually rely upon the detection of nitrite [38–45], the main impetus of the reports presented in Table 2 was nitrate determination. Those techniques that utilise nitrite as an intermediate in the determination of nitrate have been highlighted in column 3 in Table 2. The relatively inert nitrate ion is normally chemically reduced (route 1, Fig. 1) to the more reactive nitrite before initiating the detection sequence. A variety of reducing agents

have been investigated to facilitate this conversion and include zinc [19,46], amalgamated cadmium [47], hydrazine–copper [41,43] and copperised cadmium [48]. Copperised cadmium columns [48] are the most common arrangements with efficiencies for nitrate to nitrite conversion approaching 100%. A novel approach to nitrate–nitrite conversion has been the implementation of photo-induced reduction. Takeda et al. have reported the use of UV irradiation using wavelengths between

Table 2
Parameters for nitrate detection

Technique	Matrix	Direct detection of NO_3^-	Detection limit (μM)	Detection range (μM)	RSD%	FIA sample throughput/h	Reference
CE	Biological fluids	Yes	5	5–194	5.7	N/A	[6]
Greiss	Seawater	No — via NO_2^-	0.1	0–20	2	N/A	[38]
Greiss	Seawater	No — via NO_2^-	0.45	0–150	5	45	[39]
Visible	Natural water	No — via NO_2^-	0.02	0–5	1	40	[40]
Greiss	Lake water	No — via NO_2^-	0.2	0.2–10	3	240	[41]
ED	Water	No — via NO_2^-	4	7–13 600	0.91	100	[42]
UV	Water	Yes	29	29–2100	1.7	N/A	[43]
Greiss	Rain water	No — via NO_2^-	16	16–160	3	40	[43]
Greiss	Sea water	No — via NO_2^-	0.05	1–100	3	10	[44]
Griess	Aqueous	No — via NO_2^-	N/A	20–900	6	N/A	[45]
Visible	Drinking water	Yes	20	300–4000	N/A	N/A	[55]
Visible	Tap water	Yes	0.05	50–600	1	N/A	[56]
UV	Lake water	Yes	0.1	5–50	1	N/A	[57]
Visible	Aqueous	No — via NH_3	70	700–28 500	3.5	N/A	[58]
Chemiluminescence	Atmospheric	No — via NO	0.016	0.016–16	N/A	20	[80]
IR	Aqueous	Yes	N/A	100–1000	0.5	N/A	[94]
Raman	Aerosols	Yes	5	50–3000	N/A	N/A	[95]
ED	Aqueous	No — via nitration	100	500–5000	4.8	N/A	[120]
ED	Water	Yes	2.8	2.8–80	N/A	N/A	[135]
ED	Water	Yes	0.1	1–2100	9.1	N/A	[136]
ED	Sewage/water	Yes	10	10–200	4	N/A	[137]
ED	Aqueous	Yes	2	2–400	3	N/A	[143]
ED	Aqueous	Yes	0.4	1–35	4	N/A	[150]
ED	Drinking/river Water	Yes	2	5–60	1	60	[153]
ED	Drinking water	Yes	2	25–10 000	N/A	N/A	[158]
ED	Water	Yes	10	10–10 000	2.0	N/A	[159]
ED	Ground/tap water	Yes	1.5	20–10 000	N/A	N/A	[160]
ED	Carbon black	Yes	N/A	1–50	0.17	120	[161]
GC	Waste water/plants	No — via nitration	1.6	16–1600	N/A	N/A	[163]
GC	Rat urine	No — via nitration	0.5	1–1000	6.5	N/A	[164]
GC	Body fluids/serum	No — via nitration	1	1–200	N/A	N/A	[165]
GC	Rat urine	No — via nitration	1	1–1000	N/A	N/A	[165]
GC	Whole blood	Yes	10	20–1000	10	N/A	[167]
GC	Cheese/meat samples	No — via nitration	0.1	0.8–16	4.5	N/A	[169]

200 and 300 nm, resulting in the formation of nitrite and oxygen as shown below [44]. This approach is particularly attractive as it obviates the use of toxic cadmium and provides a clean and efficient alternative.



In addition, some methodologies, especially those based on chemiluminescence, require further reduction to nitric oxide (route 3, Fig. 1). A number of reducing agents have been reported to achieve this conversion, including Ti(III) [49,50], V(III) [49,51], Mo(VI) + Fe(II) and Cr(III) [49].

It is clear from the tables that a significant proportion of the reports employ some form of flow injection analysis/automation (FIA). Such assemblies are particularly amenable to the sequential analysis of both ions through the in-line incorporation of a reductor column. A schematic of a typical FIA system is shown in Fig. 2, for the indirect electrochemical detection of nitrate/nitrite (discussed in a later section), but the set-up can be easily adapted to suit the need of those protocols that require some form of derivatisation/conversion prior to conducting the actual determination. FIA techniques are highlighted in the three tables with the appropriate sample throughput; the remainder of the protocols refer to batch analysis.

The diversity of samples that have been investigated is almost matched by the protocols and techniques applied. Nevertheless, there are a number of issues that are common to all which should be highlighted before considering the vagaries of the actual analysis. While the nitrate ion is relatively inert, long-term storage of the sample prior to analysis should be discouraged through the

propensity for bacterial spoilage. This is also true for nitrite, but a number of additional precautions must be considered. Nitrite is stable in neutral or alkaline solutions but will decompose on standing in acidic conditions with the process considerably exacerbated when the solutions are heated. The redox properties of nitrite are such that many matrix constituents will act to either reduce it (e.g. ascorbic acid, sulfamic acid, urea, iodide) or oxidise it [e.g. Cr(III), Fe(III), Cu(II), MnO_4^- , CrO_4^{2-} , BrO_3^- and Ce(IV)], resulting in poor recoveries. A number of reviews have covered the effects of such interferences within food and environmental matrices [52,53].

4. Spectroscopic detection

Spectroscopic methods are by far the most widely used for nitrate/nitrite determination due to the excellent limits of detection obtained and facile assay-type protocols. A broad range of techniques have been evaluated, including UV/Vis [38–45,54–79], chemiluminescence [49,50,80,81], fluorimetric [43,54,82–93], IR [94], Raman [95] and molecular cavity emission [58,96,97] protocols. While the compositional complexities offered by real world media often preclude the direct UV determination of either nitrate or nitrite, such an approach does serve as a principal detection system in a host of chromatographic and electrophoretic systems, which are discussed in a later section. The most common approach to the detection of nitrite however is undoubtedly the Griess Assay. First developed in 1879 [98], it has found numerous applications (cf. Tables 1–3) for both nitrate and nitrite analysis. The assay typically relies on the diazotisation of a suitable aromatic amine by acidified nitrite with the subsequent coupling reaction providing a highly coloured azo chromophore from which the concentration of nitrite can be assessed (route 2, Fig. 1).

The absorption maximum for the azo product is generally in the range 500–600 nm (depending on the selected reagents) and can be detected using conventional visible spectrometry. The most common arrangement utilises sulphanilamide and *N*-(1-naphthyl)ethylenediamine [38,39,41,43,44,

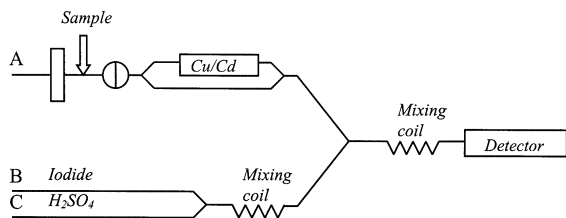


Fig. 2. FIA manifold of the flow system used in the electrochemical determination of nitrate and nitrite after reaction with iodide [117].

Table 3
Parameters for the dual detection of nitrite and nitrate

Technique	Matrix	NO ₂ ⁻ detection limit (μM)	NO ₂ ⁻ detection range (μM)	RSD%	NO ₃ ⁻ detection limit (μM)	NO ₃ ⁻ detection range (μM)	RSD%	FIA sample throughput/h	Reference
CE	Biological fluids	1	2–20	3.3	1	2–20	3.3	N/A	[7]
ED	Water/food/saliva	1.8	2.50–1000	1.75	1.7	2.50–1000	0.83	60	[46]
Chemiluminescence	Pig and dog plasma	N/A	0.4–2	N/A	N/A	0.4–2	N/A	N/A	[49]
Chemiluminescence	Water	0.01	0.01–10	0.6	0.1	0.1–100	6.7	20	[50]
UV	Water/human serum	2	1–260	N/A	3	3–400	N/A	10	[59]
Visible	Food/water	0.02	0.2–54	1.70	0.03	0.3–56	1.75	20	[60]
UV	Human plasma	0.05	0–50	6.3	N/A	0–100	2.5	N/A	[61]
UV	Rat brain tissue	0.0009	1–1000	3.4	0.0044	1–1000	3.4	N/A	[62]
UV	Food/water	1.1	0.12–240	N/A	0.81	0.083–170	N/A	N/A	[63]
ED	Food/water	1.1	0.12–24	N/A	0.81	0.83–17	N/A	N/A	[63]
Greiss	Water	0.11	0.14–2.86	0.42	0.86	0.11–2.14	0.42	N/A	[64]
Greiss	Water	0.57	0.71–18.86	0.49	0.43	0.54–14.00	0.49	N/A	[64]
Greiss	Water	0.14	0.21–7.21	0.32	0.1	0.16–5.36	0.32	N/A	[64]
UV	Biological fluids	0.1	0.2–100	5	N/A	0.2–100	5	N/A	[65]
Visible	Water samples	1.30	1.30–86.9	0.5	1.21	1.21–161	0.76	37/26	[66]
Greiss	Food/water/soil	0.02	0.22–48	2	0.16	1.6–56	2	30	[67]
Greiss	Biological fluids	1	1–300	3	1	1–300	3	30	[68]
Greiss	Biological fluids	0.025	0.025–20	N/A	0.025	0.025–20	N/A	60	[69]
Greiss	Brain tissue	0.5	1–5	1.6	0.5	1–5	1.6	40/25	[70]
Greiss	Mouse brain	0.5	1–5	1.6	0.5	1–5	1.6	40/25	[71]
Greiss	Cell cultures	0.3	N/A	N/A	0.5	N/A	N/A	N/A	[74]
Greiss	Water	0.054	N/A	2.0%	0.19	N/A	2.0	35	[75]
Greiss	Water	0.11	0–3.0	1%	0.65	0–8.7	1	N/A	[76]
Fluorescence	Water	0.028	1–100	5.0	0.052	1–100	5.0	N/A	[83]
Fluorescence	Biological samples	0.010	0.013–2.0	N/A	0.010	0.013–2.0	N/A	N/A	[92]
Fluorescence	Seawater	0.0046	N/A	N/A	0.0069	N/A	N/A	18	[93]
Visible	Meat/water	2.2	22–150	3.9	1.6	16–110	4.3	N/A	[96]
Visible	Aqueous	36	71–21 400	3	36	71–7100	10	N/A	[97]
Visible	Aqueous	71	71–14 300	5	143	143–14 300	5	N/A	[97]
AAS	Aqueous	8.7	11–220	4.7	0.65	1.6–35	3.7	35	[102]
ED	Sewage/water	5	16–200	N/A	11	16–200	N/A	N/A	[114]
ED	Human saliva	0.07	0.1–10	1.9	0.25	0.5–10	1.3	60	[117]
ED	Soil	0.4	2–1000	3	0.4	2–800	3	12	[118]
ED	Sewage/lettuce/water	12	12–200	4	10	10–200	4	N/A	[134]

Table 3 (Continued)

Technique	Matrix	NO ₂ ⁻ detection limit (μM)	NO ₂ ⁻ detection range (μM)	RSD%	NO ₃ ⁻ detection limit (μM)	NO ₃ ⁻ detection range (μM)	RSD%	FIA sample throughput/h	Reference
ED	Fertiliser	0.5	1–1000	0.42	5	10–10 000	2.0	720/60	[146]
ED	Water	0.11	0.22–22	N/A	0.081	0.16–16	N/A	N/A	[152]
ED	Human saliva	0.33	N/A	N/A	0.54	N/A	N/A	N/A	[170]
CE	Aqueous	0.73	N/A	10%	1.13	N/A	10%	N/A	[173]
CE	Vegetables	6.7	2.2–170	8.52	5.2	1.6–160	3.83	N/A	[174]
CE	Vegetables	0.74	2.2–54	4.21	0.6	1.6–26	7.37	N/A	[174]
CE	Cells	3.6	10–200	N/A	2.6	10–200	N/A	N/A	[175]
CZE	Water	0.276	N/A	3.6	0.145	N/A	6.5	N/A	[177]
CZE	Water	0.084	N/A	3.6	0.048	N/A	6.5	N/A	[177]
CZE	Water/urine	11	22–220	1	8.1	16–160	1	N/A	[178]
CZE	Seawater	1.4	N/A	1.6%	0.53	N/A	2.1%	N/A	[179]

64,68–71,99] as the target amine and coupler, respectively, with the product of the reaction detected at 540 nm. Numerous adjustments have been made to the basic procedure with assay conditions, reagents and final detection methodology being manipulated to suit given matrices. Sulphanilic acid, nitroaniline and *p*-aminoacetophenone have been used as the target amine with phenol, 1-naphthol, 1-naphthol-4-sulphonate, 1-amino naphthalene and 1,3-diaminobenzene investigated as potential coupling agents. The reaction can be used directly for the determination of nitrite, but nitrate requires the incorporation of a reduction step prior to commencing the assay.

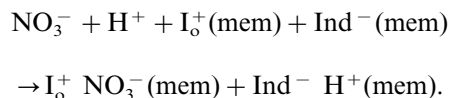
Detection limits for the Greiss assay tend to range between 0.02 and 2 μM , according to the specific reagents chosen with linearity typically spanning two orders of magnitude (1–100 μM). The technique offers a simple and effective method of detecting nitrite in a variety of matrices but the approach can suffer in complex media such as food. Antioxidants (particularly ascorbate and sulphhydryl thiols) can effectively destroy the nitrous acid before it has time to react with the indicating aromatic amine and lead to a reduction in the recovery of nitrite. The various problems that these matrices can introduce and methods to alleviate them have been reviewed previously [35]. Other problems that arise are in general those that afflict most spectroscopic determinations (i.e. highly coloured and heterogeneous matrices). HPLC and FIA have also been coupled with the Greiss protocol, to extend the diversity of samples and enable the analysis of nitrate in particularly complex matrices such as biological fluids and food samples [67–69,71].

A number of other indicating species have also given rise to coloured products. The reaction of nitrite with proflavin (3,6-diaminoacridine) in acidic conditions has been found to form a stable violet compound (λ_{max} 328 nm) with low limits of detection (2 nM) but the position of the absorption maximum can, however, increase the susceptibility to coloured interferences with Fe(III) providing significant interference at concentrations exceeding 1 mg l⁻¹ [66]. Similar problems have been encountered when the nitrosation of activated phenolics (phenol, resorcinol, phloroglu-

cinol) was used as the indicating reaction [5]. The absorption maximum could be attenuated by exploiting the ability of the resulting 1,2-hydroxy-nitroso species to coordinate with metal ions (notably Zr [100] and Cu [5]). The absorption maximum was shown to shift from 312 to 348 nm in the case of phloroglucinol/Cu. Rendering the solution basic after completion of the nitrosation steps was found to promote the greatest shift in the absorption maxima (400 nm) through the formation of the nitrosophenolate anion [5]. Devi et al. have also reported the use of anion-exchange coupled spectrophotometric detection, in which thiocyanate is displaced from a column and is subsequently reacted with Fe(III) to form a red complex that can be monitored at 480 nm, achieving detection limits of 50 nM [56].

While the majority of the UV/Vis systems employ simple assay procedures, kinetic spectroscopic protocols have also been investigated. The oxidation of gallocyanine by bromate under acidic conditions [60] occurs very slowly but is dramatically increased by the presence of nitrite. The technique relies upon measuring the decrease in the absorbance of the dye at 530 nm. Selectivity remains poor, however, as the procedure is significantly influenced by a large number of ions, particularly Fe(II), Fe(III), Ag(I), SO_3^{2-} , Br^- and I^- ions. Under the same theme, Pettas et al. have reported the use of nitrite-catalysed Thymol Blue oxidation by bromate under acidic conditions. Subsequent monitoring at 543 nm has achieved detection limits lower than 0.1 μM [72].

An optical sensor comprising a poly(vinylchloride) (PVC) membrane, impregnated with a nitrate-selective ionophore and a proton-selective (chromo)-ionophore has been developed for the continuous monitoring of nitrate in aquatic environments [55]. The potential that would arise from the complexation of nitrate ions by the ionophore (I_o) in the membrane is compensated by the co-extraction of protons by a second (chromo)-ionophore. This process is illustrated below:



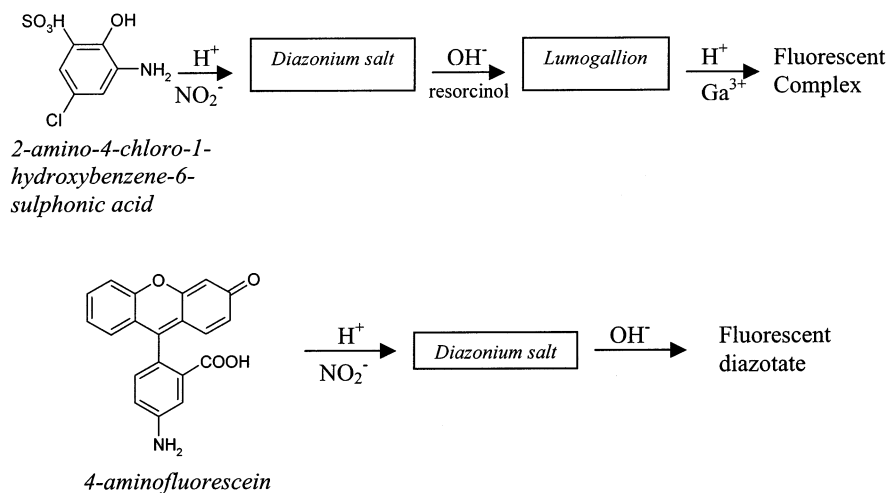


Fig. 3. Fluorimetric methods for the detection of nitrite [54]

The nitrate concentration in the solution is correlated with the amount of protons in the membrane and can be detected spectroscopically by the protonation of the indicator anion (Ind^-). In the absence of nitrate ions, the positively charged ionophore and the water-soluble indicator anion form an ion pair ($\text{I}_o^+ \text{Ind}^-$) and remain in the hydrophobic membrane. When freshly prepared, the membrane shows an intense blue colour with a λ_{max} at 612 nm. If nitrate and protons enter the membrane, the protonation of the indicator leads to a decrease in the absorption band at 612 nm. This process is reversible and can be restored to the initial absorbance by washing with buffer solution. A detection limit of 20 μM is achieved with a linear range from 300 μM to 4 mM. Despite the high detection limit, this technique has a number of advantages over potentiometric sensors in that no reference element is required, there is minimal electrical interference and baseline variation can be eliminated using multi-wavelength calibration. As in the previous situation, selectivity remains an issue in that nitrite, chloride and sulphate are potential interferences.

Fluorimetric protocols have explored a number of avenues of varying procedural complexity. Fluorimetric determination of Ce(III) resulting from the oxidation of nitrite with Ce(IV) presents one

of the more simpler processes but can be susceptible to the influence of other redox species [83]. More selective approaches have utilised the chemical versatility of acidified nitrite. The nitrosation of 4-hydroxycoumarin in acidic medium, followed by reduction results in the production of fluorescent 3-amino-4-hydroxycoumarin [82]. Lapat et al. have reported the use of two fluorometric methods that can detect nitrite when reacted with either 2-amino-4-chloro-1-hydroxybenzene-6-sulphonic acid or 4-aminofluorescein to form the diazonium salt shown in Fig. 3. The resulting solutions can be easily manipulated to produce highly fluorescent complexes [54]. Detection can be achieved at 608 nm (495 nm excitation) and 518 nm (492 nm excitation) for the respective compounds and has been successfully applied as a method of detecting the explosive 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX) after its decomposition to nitrite.

Addressing the issue of selectivity is vital when attempting an analysis on a sample that has received little pre-treatment. In the majority of the cases presented thus far, the interferences are minimised by utilising the chemical specificity of the nitrite ion to undergo nitrosation/diazotisation. This goes some way to explain the remarkable popularity of the Griess assay. However, an alternative route that also utilises the chemical versatility of nitrite has also come to the fore.

Gas-phase chemiluminescence can offer greater scope for the elimination of matrix effects in that it is based upon monitoring the reaction between gaseous NO and ozone [49,50,80,101]. Conversion of nitrite through reduction with acidified KI liberates gaseous NO from the matrix (route 3, Fig. 1). This subsequently reacts with ozone to give nitrogen dioxide in an excited state (NO_2^*) plus molecular oxygen. The excited state of NO_2^* then decays to give a weak infrared chemiluminescence above 600 nm (route 4, Fig. 1). This route can also be used for the analysis of nitrate but requires the application of stronger reductants [typically Ti(III) [49,50]]. The nitrate concentration can then be calculated by difference. The fusion of chemiluminescence with FIA assemblies can lead to significantly improved detection limits (10 nM [50,80]) over conventional UV/Vis systems and can also facilitate high-throughput sample analysis with the quantitative determination of nitrate, nitrite and ammonia requiring less than 3 minutes. A significant drawback to the protocol lies in the increased technical complexity of the system and the requirement of high combustion temperatures (600°C). This is due to the reaction of trace oxygen with NO forming NO_2 , resulting in a decrease in chemiluminescence intensity. At high temperatures (600°C), the re-conversion of NO_2 to NO increases, compensating for the interference caused by oxygen.

Molecular emission cavity analysis (MECA) continues along a similar vein with particular applicability to the analysis of highly coloured solutions that would prove intractable for standard spectroscopic techniques. Nitrite is again reduced to NO and swept into the flame by nitrogen carrier gas. The resulting emission is then monitored at 640 nm. Spectral interferences from carbon dioxide and hydrogen sulphide, which have been found to emit within the cavity, can be problematic, although the latter could be alleviated through precipitation as a suitable metallic sulphide.

The remainder of the spectroscopic systems tend to deviate from mainstream detection and have been used largely for a number of specialised tasks but have been included for the sake of completeness. Fourier-transform infrared spec-

troscopy (FT-IR) and Raman spectroscopy have been utilised for the spectroscopic determination of the nitrate ion in aerosols [12,94,95]. The former measures the absorbance bands at 1384 and 2430 cm^{-1} , which are attributed to the nitrate ion [12]. Morphology-dependent stimulated Raman scattering (MDSRS) is a non-linear spectroscopy that uses the set of 'macroscopic' natural electromagnetic modes of oscillation characteristics of micrometre-sized axisymmetric particles to enhance Raman scattering from molecules contained within the particle. The modes of oscillation are commonly called morphology-dependent resonances (MDRs) because their spatial and spectral positions are determined by particle size, shape and refractive index. The detection limits are of a low micromolar order with the dynamic range extending to 3 mM [95].

Gallego et al. have reported the indirect determination of nitrate and nitrite using atomic absorption spectroscopy coupled with FIA (FIA-AAS) [102]. Nitrite and nitrate form ion pairs with copper(I)-neocuproine chelate that are then extracted into methyl-isobutyl-ketone, where the atomic-absorption signal of copper from the organic phase is proportional to the nitrate or nitrite concentration. The reported detection limits are 8.7 and $0.65\text{ }\mu\text{M}$ for nitrite and nitrate, respectively, at a sample throughput of 35/h. Interferences are evident from thiocyanate, perchlorate and chlorate ions. Wennmalm et al. have reported the use of nitrite detection using electron paramagnetic resonance spectrometry (EPR) [103]. The system is based on measuring the paramagnetic properties of NO bound to heme-containing proteins, which can be used for quantitative determination in the nanomolar range.

5. Electrochemical detection

The electrochemical detection of nitrate and nitrite can be divided into a number of categories. Fortunately, these can be broadly grouped within the distinctions of voltammetric and potentiometric systems. Voltammetric techniques have been employed from the beginning of the 1900s,

whereby copper electrodes were used to reduce the nitrate ion [104–111] electrochemically. A wide variety of electrode substrates have since been investigated and include: nickel [112,113], copper–nickel alloys [114], cadmium [105,115,116], platinum [117,118], glassy carbon [119,120], gold [121], lead [112,122], silver [110,123] and, more recently, boron-doped diamond [124–126]. The profusion of electrode materials effectively betrays the fact that the electroanalytical determination of nitrate and nitrite at bare electrodes is far from facile. Despite the thermodynamic feasibility of the reduction, the kinetics of the charge transfer are slow [127] and, as such, direct reduction of nitrate has been characterised by poor sensitivity and often marked irreproducibility through cumulative electrode passivation effects. The need to apply large overpotentials can also affect the selectivity of the approach.

Nitrite would appear to be rather more fortunate in that this ion can be oxidised or reduced at a glassy carbon electrode [128–131]. Unfortunately, neither option is particularly favourable in terms of direct electroanalysis, succumbing to similar problems that afflict nitrate analysis [127,130–132]. A number of routes, outlined below, have been pursued in an attempt to counter these problems of which electrode modification appears to offer the most benefits in terms of sensitivity and selectivity. Immobilised electrocatalysts can offer marked improvements in selectivity and sensitivity (particularly those based on biorecognition). Surface modifications are often perceived as increasing the technical and procedural complexity of the protocol, and while this is certainly true when considering the immobilisation of enzymes, much simpler options exist for enhancing the reduction of both nitrate and nitrite.

Increasing the sensitivity of the electrode response can be achieved through maintaining a large and highly active surface area. This has traditionally been attempted [109,111,116,133] through the deposition of a fresh electrode layer prior to each analysis. This can be done simply by the introduction of the appropriate metal salt(s) into the sample medium. The analysis is per-

formed by sweeping a potential range in the cathodic direction. The metal ion (typically Cu [109] or Cu/Cd [116,133] mixtures) is electrolytically plated onto the electrode and effectively provides a fresh surface from which nitrate or nitrite reduction is induced as the potential scan progresses. A significant advantage of this approach is that the analysis is relatively independent of the base electrode material as the nitrate/nitrite reduction occurs at the freshly deposited metal layer. This has been exploited in the development of disposable capillary fill devices in which screen printed carbon electrodes were used in conjunction with cupric sulphate to provide a response to nitrate [111].

An alternative approach is to prepare the electrode *ex situ* whereby the electrode surface is conditioned in a plating solution of defined composition and then transferred to the analysis solution [134–136]. This approach offers greater control over the morphological features of the deposit, which, in the case of a cupric ion/sulphate/chloride mixture, was found to lead to a particularly active granular deposit. Whilst this approach will still fall foul of cumulative passivation effects, it was found to retain substantial activity over a 24 h period of continued use. More recently, electrode cleaning and activation have been achieved through the application of 20 kHz ultrasound, extending the sample diversity of nitrate/nitrite detection to highly passivating matrices such as whole egg [119] or sewage [137]. Sono-electroanalytical techniques provide a novel on-site method of analysis precluding the requirement for sample clean-up.

More complicated surface modifications have also tended to make use of metal ions though usually bound within some form of complex [138]. Electrocatalysts used for the reduction of nitrate and nitrite include: alternating layers of μ -meso (tetrapyrrolyl)porphyrinate Co(III) tetrakis [bis(bipyridine)(chloro) Ru(II)] and meso-tetra(4-sulphonatophenyl)porphyrinate Zn(II) [139], polypyrrole films doped with tungstodiphosphate anion ($P_2W_{18}O_{62}^{6-}$) [140], P–Mo–V heteropolyacid [141] or iron-substituted heteropolytungstates [142] and Nafion-coated mercury film electrodes incorporating Yb^{3+} or UO_2^{2+} [143]. The ability to

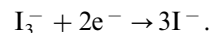
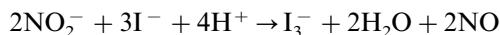
electrochemically oxidise nitrite has not gone unnoticed, and a number of electrode assemblies based upon metal ions (typically Os [42], Ru [144–146] or Ir [147]) coordinated to, or electrostatically entrapped within, polyvinyl pyridine supports have also been investigated. Other techniques utilise the reduction of nitrite to NO, which can then be detected amperometrically at modified Au electrodes covered with a PTFE film [148].

The more sophisticated approaches to the electrochemical detection of nitrate and nitrite probably lie with the use of biological catalysts. Reductase enzymes [149,150] can significantly enhance both electrode sensitivity and selectivity towards the reduction of nitrate and nitrite respectively. Reshetilov et al. have proposed the use of a biosensor based on the oxidation of nitrite by bacteria based on the *N. vulgaris* strain [151]. The nitrite oxidation is then followed using amperometric methods for oxygen content measurement, which is released as a by-product of the reaction with nitrite. Despite these advantages, the high reagent expense combined with the fragility and complexity of the sensing layer has, as yet, tended to preclude the widespread acceptance of this route.

A large number of assay type protocols for the detection of nitrate and more commonly nitrite have arisen as a consequence of the difficulties experienced in the direct electroanalysis of these ions. As with the previous spectroscopic assays, nitrate determination is usually accomplished through its reduction to nitrite using a Cu/Cd column. There are, however, a number of procedures that do in fact utilise the chemical properties of nitrate — principally, its ability to nitrate aromatics such as benzoic acid, salicylic acid, isoquinoline and thiophene-2-carboxylic acid (route 5, Fig. 1). The resulting nitro derivative is determined reductively at a glassy carbon electrode, using linear sweep voltammetry (LSV) between 0 and -0.5 V. Of the compounds studied, thiophene-2-carboxylic acid gave the best electrochemical response [120]. A similar approach has been developed in conjunction with HPLC techniques whereby the nitration of phenol is the principal indicating reaction with the analytical

signal provided through the reduction of *o*-nitrophenol at -0.47 V [152]. In comparison with the thiophene-2-carboxylic acid nitration, 1000-fold higher detection limits are achieved ($0.1 \mu\text{M}$) with fewer problems reported from interferences, such as dissolved oxygen [120]. Nitrate has also been shown to oxidise uranyl ions, which can then be reduced catalytically at an electrode surface and used as an indirect method of detection with detection limits of $2 \mu\text{M}$ [153].

Indirect methods for nitrite analysis are slightly more diverse given the greater versatility of this ion. An electrochemical variation/adaptation of the Griess assay has been attempted with the reduction of the diazonium salts resulting from the reaction of nitrous acid with substituted phenylene diamines providing the analytical signal. The nitrosation of activated phenolic compounds can open up another route with the reduction of the nitroso group to the corresponding amine providing a sensitive response within a region (~ -0.2 V) where there are few electroactive interferences [5,154]. The iodine–iodide couple is a reversible electrochemical system on both gold and platinum electrodes in sulphuric acid media [155], which can be exploited for indirect analysis of nitrite. The technique is based on the reaction of nitrite with iodide in acidic medium to form triiodide, which is then amperometrically monitored via triiodide reduction between $+0.2$ and $+0.3$ V (vs. SCE).



This approach has been studied at Au and Pt macroelectrodes [156] and also at a Pt microelectrode [117]. This technique is relatively simple, quick and easy, and can be combined with a flow injection analysis (FIA) protocol — giving analytical throughput in the region of 60 samples h^{-1} . When coupled with FIA (Fig. 2), detection limits as low as 70 nM nitrite have been established [117]. Biamperometric detection of the iodine/triiodide system has also been employed in a flow-through cell using two teflonized graphite or platinum electrodes. This set-up offers advantages in experimental simplicity due to lower re-

duction potentials required (100 mV) [118]. The main problem with using the reaction of nitrite with iodide is interference by oxygen, which can oxidise both I^- and NO , and therefore, stringent use of degassed solutions is required.

In addition to the numerous amperometric and voltammetric detection protocols previously mentioned, potentiometric methods for nitrate/nitrite analysis are also routinely available, further extending the diversity of electrochemical analytical methods. The most common approach is the use of commercially available ion-selective electrodes (ISEs), whereby the selective passage of charged species from one phase to another, typically from solution to membrane, gives rise to a potential difference, which varies with a Nernstian fashion with the activity of the ionic species, in this case NO_3^- , so that a calibration graph of potential versus nitrate concentration can be obtained. Several authors acknowledge the widespread use of a PVC membrane [157–162] used in conjunction with an ion-exchanger and plasticizer. In modern times, the use of both tetradodecylammonium bromide (TDABr) [157] and tetradodecylammonium nitrate (TDAN) [159,160] as ion exchangers and the use of dibutylphthalate (DBP) [157,159] and *o*-nitrophenyl octyl ether (*o*-NPOE) [158,160,161] as plasticizers have been reported. Detection limits for nitrate offer micromolar resolution coupled with good dynamic ranges spanning from 10^{-5} to 10^{-2} M. Direct detection of nitrite has been reported when coupled with ion chromatographic separation methods [157]. ISEs offer attractive features as nitrate sensors, including the ease with which they can be coupled with continuous flow systems or in flow systems with

sample injection, such as FIA, enhancing sample diversity. Excellent longevity is commonly observed, with in-situ operation lasting in excess of 15 months [160]. Interference from other anions is often minimal by virtue of the low selectivity coefficients (10^{-3}) achieved.

6. Chromatography

Most branches of chromatography have been utilised in the search for technologies capable of detecting nitrate and nitrite. Derivatisation protocols are a necessity for many (particularly gas chromatography), but direct sample introduction can be accomplished with relative ease in most HPLC and ion chromatographic systems. In most cases, some form of sample pre-treatment is required whether it be simple filtration or derivatisation, and it is these stages that tend to remove the attractiveness of the technique. One of the more common derivatisation procedures is the conversion of nitrate to the electrophilic nitrosonium ion (NO_2^+) with the subsequent nitration of an appropriate activated aromatic (2,4-dimethylphenol [163] and 1,3,5-trimethoxybenzene [28]) providing the indicating species (route 5, Fig. 1) [164,165]. Nitrite can be similarly determined through prior oxidation to nitrate with weak hydrogen peroxide. An alternative derivatisation protocol for nitrite that also involves the formation of a nitro species is by nucleophilic substitution with an aliphatic halide. Pentafluorobenzyl bromide (PFB) is particularly adept at scavenging nitrite ions to form the corre-

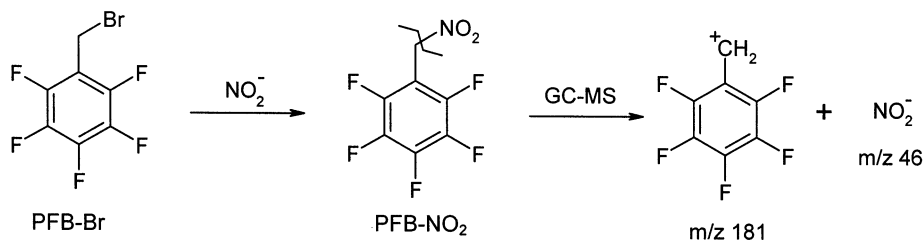


Fig. 4. GC-MS detection of nitrite via nucleophilic substitution with pentafluorobenzyl bromide (PFB-Br) [166]

sponding nitro derivative as shown in Fig. 4. Analysis by GC-MS results in the weak methylene-nitro bond being fragmented and the NO_2^- ion detected unambiguously [166,167]. This technique can also be extended to incorporate ^{15}N detection, after reaction of the aromatic with labelled nitrate/nitrite. An alternative derivatisation agent for nitrite is the use of *N*-acetyl-L-cysteine with the production of S-nitroso-*N*-acetyl-L-cysteine [61]. The exploitation of such a route is quite ironic given that sulphhydryl thiols are often the source of poor nitrite recoveries in food matrices [52].

End column detection systems include simple UV [28,57,61,65,168], fluorimetric [83], electron capture [169], electrochemical [62,63,152,170] and mass spectroscopic [9,166] analysis of the eluent. The popularity of UV detection lies in the simplicity of the approach and the ability to attenuate the detection wavelength. The low absorbance of most inorganic ions helps to maintain a low background from which nitrite and nitrate can be resolved. However, the aromatic eluents typically used in anion exchange tend to give rise to high background UV absorbance, complicating the direct detection of nitrate/nitrite. Alkane-sulphonates, which are eluents with a low background UV absorbance, have proven to be useful alternatives in this respect [168]. In contrast, Grosjean has studied the use of “negative” UV detection whereby a highly absorbing eluent is used (e.g. aromatic acid) in the HPLC [171]. In this way, analytes that absorb only weakly are recorded as ‘negative’ peaks. This ‘indirect photometry’ allows for substantially better detection limits than that normally achieved for direct methods for weakly absorbing anions.

Electrochemical detection is normally reserved for nitrite through oxidation at glassy carbon electrodes (typically poised between +0.7 and +0.9 V) [62,63]. Nitrate can also be detected amperometrically but requires exposure to UV radiation prior to reaching the electrochemical detector. The photolysis product has been shown to be capable of oxidation in a manner analogous to that of nitrite. Excellent detection limits of 0.9 and 4.4 nM were obtained for nitrite and nitrate, respectively [62].

7. Capillary electrophoresis

Capillary electrophoresis (CE) is a powerful separation technique first reviewed for clinical use in 1995 [172], but which has rapidly been transformed for more general analytical purposes [6,7,173–175]. The main advantages of this technique lie in the possibility of fast simultaneous detection of a wide variety of anions. Other attractive features of CE are small sample requirements (nl) and low buffer consumption, in comparison with HPLC. In addition, the instrumentation has automated sample loading, requires little maintenance and is arguably more cost-effective than other methods. A more recent development of CE is the use of capillary zone electrophoresis (CZE), which has increased the sensitivity of the technique by a factor of 10, giving 0.1 μM limits of detection [176–179]. Detection of nitrate and nitrite is normally performed by UV detection at 214 nm. While CE can be directly applied to a number of matrices, a number of preparative/separation stages are usually required to remove proteinaceous material that would otherwise exert a detrimental influence on the both the analytical signal and the actual equipment through adsorption on the wall of the capillary. The addition of deproteinization steps can increase dramatically the lifetime of the system to 250–300 determinations without significant change in retention times [7].

8. Conclusions

The aim of this review was to explore the vast diversity of methods currently available to the reader for the detection and analysis of nitrate and/or nitrite in a variety of sample matrices. Awareness has been drawn to the benefits and drawbacks of each technique in an attempt to provide a general evaluation of this broad scientific area of study. Where possible, the review has focused on the more contemporary techniques of the past decade in an attempt to offer a more modern and up-to-date account of nitrate/nitrite analytical strategies. In summary, the use of spectroscopic analysis appears to be the method of

choice due to the simplicity of the protocols and the wide availability of the instrumentation involved. The increasing demand for rapid on-site analysis will ensure the continued development of both spectroscopic and electrochemical methods, which are more applicable to miniaturisation and remote operation. It could be envisaged that while chemiluminescent and chromatographic techniques offer superior performance, the instrumental complexity required for their successful implementation will limit their operation to a laboratory-based environment.

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References

- [1] P.F. Swann, *J. Sci. Food. Agric.* 26 (1975) 1761.
- [2] C.S. Bruning-Fann, J.B. Kaneene, *Vet. Human. Toxicol.* 35 (1993) 521.
- [3] Water Supply Regulations, Statutory Instruments 1989, No. 1147, HMSO, London, 1989.
- [4] EC Draft Regulation (VI/3080/93) Revision 7.
- [5] J. Davis, K.J. McKeegan, M.F. Cardosi, D.H. Vaughan, *Talanta* 50 (1999) 103.
- [6] M.A. Friedberg, M.E. Hinsdale, Z.K. Shihabi, *J. Chromatogr. A* 781 (1997) 491.
- [7] P.N. Bories, E. Scherman, L. Dziedzic, *Clin. Biochem.* 32 (1999) 9.
- [8] G. Ellis, I. Adatia, M. Yazdanpanah, S.K. Makela, *Clin. Biochem.* 31 (1998) 195.
- [9] D. Tsikas, I. Fuchs, F.M. Gutzki, J.C. Frölich, *J. Chromatogr. B* 715 (1998) 441.
- [10] J.B. Fox, *CRC Crit. Rev. Anal. Chem.* 15 (1985) 283.
- [11] L.H.J.M. Janssen, H. Visser, F.G. Römer, *Atmos. Environ.* 23 (1989) 2783.
- [12] J.S. Bogard, S.A. Johnson, R. Kumar, P.T. Cunningham, *Environ. Sci. Technol.* 16 (1982) 136.
- [13] K. Yoshizumi, K. Aoki, *Anal. Chem.* 57 (1985) 737.
- [14] R.P. Wayne, I. Barnes, P. Biggs, J.P. Burrows, C.E. Canosa-Mas, J. Hjorth, G. Le Bras, G.K. Moortgat, D. Perner, G. Poulet, G. Restelli, H. Sidebottom, *Atmos. Environ.* 25A (1991) 1.
- [15] M.D. King, E.M. Dick, W.R. Simpson, *Atmos. Environ.* 34 (2000) 685.
- [16] United Nations Environment Programme (UNEP) Millennium Report on the Environment, Global Environmental Outlook, Earthscan Publications, London, 1999.
- [17] P. Brimblecombe, D.H. Stedman, *Nature* 298 (1982) 460.
- [18] H. Moller, J. Landt, E. Pederson, P. Jensen, H. Autrup, O. Moller Jensen, *Cancer Res.* 49 (1989) 3117.
- [19] M.A. Koupparis, K.M. Walczak, H.V. Malmstadt, *Anal. Chim. Acta* 142 (1982) 119.
- [20] D. Forman, S. Al-Dabbagh, R. Doll, *Nature* 313 (1972) 620.
- [21] M. Yamaguchi, S. Itakura, *Fish. Sci.* 65 (1999) 367.
- [22] European Standards for Drinking Water, second ed., World Health Organisation, Geneva, 1970, p. 36.
- [23] A.D. Eaton, L.S. Clescheri, A.E. Greenberg, *Standard Methods for the Examination of Water and Wastewater*, 16th ed., American Public Health Association, New York, 1978.
- [24] R.D. Cox, C.W. Frank, *J. Anal. Toxicol.* 6 (1982) 148.
- [25] M.A. Kuiper, J.J. Visser, P.L. Bergmans, P. Scheltens, E.C. Wolters, *J. Neurol. Sci.* 121 (1994) 46.
- [26] R.M.J. Palmer, A.G. Ferrige, S. Moncada, *Nature* 327 (1987) 524.
- [27] P.C. Ford, D.A. Wink, D.M. Stanbury, *FEBS Lett.* 326 (1993) 1.
- [28] D. Tsikas, F.M. Gutzki, J.C. Frölich, *Fres. J. Anal. Chem.* 342 (1992) 95.
- [29] A.A. Johnson, D.G. Burleson, *Anal. Biochem.* 236 (1996) 331.
- [30] E. Culotta, D.E. Koshland, *Science* 258 (1992) 1862.
- [31] D. Tsikas, F.M. Gutzki, S. Rossa, H. Bauer, C. Neumann, K. Dockendorff, J. Sandmann, J.C. Frölich, *Anal. Biochem.* 244 (1997) 208.
- [32] J.B. Ochoa, A.O. Udekwu, T.R. Billar, R.D. Curran, F.B. Cerra, R.L. Simmons, A.B. Peitzman, *Ann. Surg.* 214 (1991) 621.
- [33] P.S. Grabowski, A.J. England, R. Dykhuizen, M. Copland, N. Benjamin, D.M. Reid, S.H. Ralston, *Arthritis Rheum.* 39 (1996) 643.
- [34] R.F. Kornelisse, K. Hoekman, J.J. Visser, W.C.J. Hop, J.G. Huijman, P.J. Straaten, A.J. Heijden, R.N. Sukhai, H.J. Neijens, R. Groot, *J. Infect. Dis.* 174 (1996) 120.
- [35] H. Trachtman, B. Gauthier, R. Frank, S. Futterweit, A. Goldstein, J. Tomczak, *J. Pediatr.* 128 (1996) 173.
- [36] S.T. Davidge, C.P. Stranko, J.M. Roberts, *Am. J. Obstet. Gynecol.* 174 (1996) 1008.
- [37] Y. Ueki, S. Miyake, Y. Tominaga, K. Eguchi, *J. Rheumatol.* 23 (1996) 230.
- [38] H.W. Jannasch, K.S. Johnson, C.M. Sakamoto, *Anal. Chem.* 66 (1994) 3352.
- [39] A. Daniel, D. Birot, M. Lahaitre, J. Poncin, *Anal. Chim. Acta* 308 (1995) 413.
- [40] S. Nakashima, M. Yagi, M. Zenki, A. Takahashi, K. Tōei, *Fres. Z. Anal. Chem.* 319 (1984) 506.
- [41] J. Hilton, E. Rigg, *Analyst* 108 (1983) 1026.
- [42] M.A. Stanley, J. Maxwell, M. Forrestal, A.P. Doherty, B.D. MacCraith, D. Diamond, J.G. Vos, *Anal. Chim. Acta* 299 (1994) 81.

- [43] B.C. Madsen, *Anal. Chim. Acta* 124 (1981) 437.
- [44] K. Takeda, K. Fujiwara, *Anal. Chim. Acta* 276 (1993) 25.
- [45] H. Borchering, S. Leikefeld, C. Frey, S. Diekmann, P. Steinrucke, *Anal. Biochem.* 282 (2000) 1.
- [46] X.L. Su, P. Chen, X.G. Qu, W.Z. Wei, S.Z. Yao, *Microchem. J.* 59 (1998) 341.
- [47] Z. Marczenko, *Spectrophotometric Determination of Elements*, Ellis Horwood, Chichester, UK, 1976, pp. 397–399.
- [48] J.F. Van Staden, *Anal. Chim. Acta* 138 (1982) 403.
- [49] F. Yang, E. Troncy, M. Francœur, B. Vinet, P. Vinay, G. Czaika, G. Blaise, *Clin. Chem.* 43 (1997) 657.
- [50] T. Aoki, S. Fukuda, Y. Hosoi, H. Mukai, *Anal. Chim. Acta* 349 (1997) 11.
- [51] R.S. Braman, S.A. Hendrix, *Anal. Chem.* 61 (1989) 2715.
- [52] J.B. Adams, *Food Chem.* 59 (1997) 401.
- [53] J.C. Fanning, *Coord. Chem. Rev.* 199 (2000) 159.
- [54] A. Lapat, L. Székelyhidi, I. Hornyák, *Biomed. Chromatogr.* 11 (1997) 102.
- [55] R. Lump, J. Reichert, H.J. Ache, *Sensors and Actuators B* 7 (1992) 473.
- [56] S. Devi, A. Townshend, *Anal. Chim. Acta* 225 (1989) 331.
- [57] D.C. Schroeder, *J. Chromatographic Science* 25 (1987) 405.
- [58] R. Belcher, S.L. Bogdanski, A.C. Calokerinos, A. Townshend, *Analyst* 106 (1981) 625.
- [59] I.N. Papadoyannis, V.F. Samanidou, C.C. Nitsos, *J. Liq. Chromatogr. R.T.* 22 (1999) 2023.
- [60] A.A. Ensafi, A. Kazemzadeh, *Anal. Chim. Acta* 382 (1999) 15.
- [61] D. Tsikas, S. Rossa, J. Sandmann, J.C. Frölich, *J. Chromatogr. B* 724 (1999) 199.
- [62] V. Rizzo, L. Montalbetti, A.L. Rozza, W. Bolzani, C. Porta, G. Balduzzi, E. Scoglio, R. Moratti, *J. Chromatogr. A* 798 (1998) 103.
- [63] M. Lookabaugh, I.S. Krull, *J. Chromatogr.* 452 (1988) 295.
- [64] K. Horita, G.F. Wang, M. Satake, *Analyst* 122 (1997) 1569.
- [65] S.A. Everett, M.F. Dennis, G.M. Tozer, V.E. Prise, P. Wardman, M.R.L. Stratford, *J. Chromatogr. A* 706 (1995) 437.
- [66] R.S. Guerrero, C.G. Benito, J.M. Calatayud, *Talanta* 43 (1996) 239.
- [67] M.J. Ahmed, C.D. Stalikas, S.M. Tzouwara-Karayanni, M.I. Karayannis, *Talanta* 43 (1996) 1009.
- [68] L.C. Green, D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnok, S.R. Tannenbaum, *Anal. Biochem.* 126 (1982) 131.
- [69] P.F. Pratt, K. Nithipatikom, W.B. Campbell, *Anal. Biochem.* 231 (1995) 383.
- [70] I. Yokoi, H. Habu, H. Kabuto, A. Mori, *Meth. Enzymol.* 268 (1996) 152.
- [71] H. Habu, I. Yokoi, H. Kabuto, A. Mori, *Neurochemistry* 5 (1994) 1571.
- [72] I.A. Pettas, S.I. Lafis, M.I. Karayannis, *Anal. Chim. Acta* 376 (1998) 331.
- [73] Y. Fang, T. An, X. Shi, X. Jin, H. Chen, Xibei Shifan Daxue Xuebao, *Ziran Kexueban* 36 (2000) 39.
- [74] L.A. Ridnour, J.E. Sim, M.A. Hayward, D.A. Wink, S.M. Martin, G.R. Buettner, D.R. Spitz, *Anal. Biochem.* 281 (2000) 223.
- [75] H. Chen, Y. Fang, T. An, K. Zhu, J. Lu, *Int. J. Environ. Anal. Chem.* 76 (2000) 89.
- [76] M.N. Abbas, G.A. Mostafa, *Anal. Chim. Acta* 410 (2000) 185.
- [77] M. Miro, A. Cladera, J.M. Estela, V. Cerda, *Analyst* 125 (2000) 943.
- [78] M. Barzegar, M.F. Mousavi, A. Nemati, *Microchem. J.* 65 (2000) 159.
- [79] G.M. Greenway, S.J. Haswell, P.H. Petsul, *Anal. Chim. Acta* 387 (1999) 1.
- [80] K. Yoshizumi, K. Aoki, *Anal. Chem.* 57 (1985) 737.
- [81] Z.K. He, B. Fuhrmann, U. Spohn, *Fres. J. Anal. Chem.* 367 (2000) 264.
- [82] T. Ohta, Y. Arai, S. Takitani, *J. Pharmaceut. Sci.* 76 (11) (1987) 531.
- [83] S.H. Lee, L.R. Field, *Anal. Chem.* 56 (1984) 2647.
- [84] Z. Huang, T. Korenaga, M.I.H. Helaleh, *Mikrochim. Acta* 134 (2000) 179.
- [85] A. Buldt, U. Karst, *Anal. Chem.* 71 (1999) 3003.
- [86] H. Wang, W. Yang, S.C. Liang, Z.M. Zhang, H.S. Zhang, *Anal. Chim. Acta* 419 (2000) 169.
- [87] B. Yuan, Q. Lin, *Fenxi Huaxue* 28 (2000) 692.
- [88] N.Q. Jie, D.L. Yang, Q.B. Jiang, Q. Zhang, L. Wei, *Microchem. J.* 62 (1999) 371.
- [89] M. Wada, M. Kurose, A. Nakamura, N. Kuroda, M. Tanigawa, K. Nakashima, *Nippon Kasei Gakkaishi* 51 (2000) 115.
- [90] K. Geetha, N. Balasubramanian, *Anal. Lett.* 33 (2000) 1869.
- [91] M.I.H. Helaleh, T. Korenaga, *Microchem. J.* 64 (2000) 241.
- [92] H. Li, C.J. Meininger, G.Y. Wu, *J. Chromatogr. B* 746 (2000) 199.
- [93] R.T. Masserini, K.A. Fanning, *Mar. Chem.* 68 (2000) 323.
- [94] G. Jiao, S.H. Lips, *J. Plant Nutr.* 23 (2000) 79.
- [95] P.M. Aker, J. Zhang, W. Nichols, *J. Chem. Phys.* 110 (1999) 2202.
- [96] A. Celik, E. Henden, *Analyst* 114 (1989) 563.
- [97] I.Z. Al-Zamil, A. Townshend, *Anal. Chim. Acta* 142 (1982) 151.
- [98] J.P. Greiss, *Ber. Dtsch. Chem. Ges.* 12 (1879) 426.
- [99] M.F. Giné, B.F. Reis, E.A.G. Zagatto, F.J. Krug, A.O. Jacintho, *Anal. Chim. Acta* 155 (1983) 131.
- [100] J. Gabbay, Y. Almog, M. Davidson, A.E. Donagi, *Analyst* 102 (1997) 371.
- [101] R.D. Cox, *Anal. Chem.* 52 (1980) 332.

- [102] M. Gallego, M. Silva, M. Valcárcel, *Fres. Z. Anal. Chem.* 323 (1986) 50.
- [103] A. Wennmalm, A. Petersson, *J. Cardiovasc. Pharmacol.* 17 (3) (1991) S34.
- [104] A. Turrentine, *Trans. Am. Electrochem. Soc.* 10 (1896) 49.
- [105] S. Glasstone, A. Hickling, *Electrolytic Oxidation and Reduction: Inorganic and Organic*, Chapman Hall, London, 1934.
- [106] D. Pletcher, Z. Poorabedi, *Electrochim. Acta* 24 (1979) 1253.
- [107] M. Shibata, K. Yoshida, N. Furuya, *J. Electroanal. Chem.* 387 (1995) 143.
- [108] M. Shibata, K. Yoshida, N. Furuya, *J. Electrochem. Soc.* 145 (1998) 2348.
- [109] N.G. Carpenter, D. Pletcher, *Anal. Chim. Acta* 317 (1995) 287.
- [110] S. Cattarin, *J. Appl. Electrochem.* 22 (1992) 1077.
- [111] A.G. Fogg, S.P. Scullion, T.E. Edmonds, B.J. Birch, *Analyst* 116 (1991) 573.
- [112] H.L. Li, J.Q. Chambers, D.T. Hobbs, *J. Appl. Electrochem.* 18 (1988) 454.
- [113] J.O. Bockris, J. Kim, *J. Electrochem. Soc.* 143 (1996) 3801.
- [114] M.J. Moorcroft, L. Nei, J. Davis, R.G. Compton, *Anal. Lett.* 33 (2000) 3127.
- [115] R.J. Davenport, D.C. Johnson, *Anal. Chem.* 45 (1973) 1979.
- [116] M.E. Bodini, D. Sawyer, *Anal. Chem.* 49 (1977) 485.
- [117] V. Mori, M. Bertotti, *Anal. Lett.* 32 (1999) 25.
- [118] A. Hulanicki, W. Matuszewski, M. Trojanowicz, *Anal. Chim. Acta* 194 (1987) 119.
- [119] J. Davis, R.G. Compton, *Anal. Chim. Acta* 404 (2000) 241.
- [120] A.G. Fogg, S.P. Scullion, T.E. Edmonds, *Analyst* 114 (1989) 579.
- [121] T. Ohmori, M.S. El-Deab, M. Osawa, *J. Electroanal. Chem.* 470 (1999) 46.
- [122] J.D. Genders, D. Hartsough, D.T. Hobbs, *J. Appl. Electrochem.* 26 (1996) 1.
- [123] M. Fedurco, P. Kedzierzawski, J. Augustynski, *J. Electrochem. Soc.* 146 (1999) 2569.
- [124] F. Bouamrane, A. Tadjeddine, J.E. Butler, R. Tenne, C. Levy-Clement, *J. Electroanal. Chem.* 405 (1996) 95.
- [125] C. Reuben, E. Galun, H. Cohen, R. Tenne, R. Kalish, Y. Muraki, K. Hashimoto, A. Fujishima, J.M. Butler, C. Levy-Clement, *J. Electroanal. Chem.* 396 (1995) 233.
- [126] R. Tenne, K. Patel, K. Hashimoto, A. Fujishima, *J. Electroanal. Chem.* 347 (1993) 409.
- [127] Z. Zhao, X. Cai, *J. Electroanal. Chem.* 252 (1988) 361.
- [128] A.Y. Chamsi, A.G. Fogg, *Analyst* 113 (1988) 1723.
- [129] J.E. Newburg, M.P. Lopez de Haddad, *Analyst* 110 (1985) 81.
- [130] D.L. Ehman, D.T. Sawyer, *J. Electroanal. Chem.* 16 (1963) 541.
- [131] W.M. Graven, *Anal. Chem.* 31 (1959) 1197.
- [132] J.A. Cox, P.J. Kulesza, *J. Electroanal. Chem.* 175 (1984) 105.
- [133] G.A. Sherwood, D.C. Johnson, *Anal. Chim. Acta* 129 (1981) 87.
- [134] J. Davis, M.J. Moorcroft, S.J. Wilkins, R.G. Compton, M.F. Cardosi, *Analyst* 125 (2000) 737.
- [135] A.O. Solak, P. Gulser, E. Gokmese, F. Gokmese, *Turk. Mikrokim. Acta* 134 (2000) 77.
- [136] G.A. Sherwood, D.C. Johnson, *Anal. Chim. Acta* 129 (1981) 101.
- [137] J. Davis, M.J. Moorcroft, S.J. Wilkins, R.G. Compton, M.F. Cardosi, *Electroanalysis*, 12 (2000) 1367.
- [138] A.P. Doherty, R.J. Forster, M.R. Smyth, J.G. Vos, *Anal. Chim. Acta* 255 (1991) 45.
- [139] C.M.N. Azevedo, K. Araki, L. Angnes, H.E. Toma, *Electroanalysis* 10 (1998) 467.
- [140] Z. Liu, X. Xi, S. Dong, E. Wang, *Anal. Chim. Acta* 345 (1997) 147.
- [141] M. Wu, S. Wang, *Huaxue Fence* 36 (2000) 244.
- [142] B. Fabre, G. Bidan, M. Lapkowski, *J. Chem. Soc. Chem. Commun.* 12 (1994) 1509.
- [143] P. Ugo, L.M. Moretto, B. Ballarin, *Electroanalysis* 7 (1995) 129.
- [144] T.J. O'Shea, D. Leech, M.R. Smyth, J.G. Vos, *Talanta* 39 (1992) 443.
- [145] J.N. Barisci, G.G. Wallace, *Anal. Lett.* 24 (1991) 2059.
- [146] A.P. Doherty, M.A. Stanley, D. Leech, J.G. Vos, *Anal. Chim. Acta* 319 (1996) 111.
- [147] J.A. Cox, K.R. Kulkarni, *Analyst* 111 (1986) 1219.
- [148] H. Kobayashi, M. Hikuma, *Anal. Lett.* 33 (2000) 1013.
- [149] I. Willner, A. Riklin, N. Lapidot, *J. Am. Chem. Soc.* 112 (1990) 6438.
- [150] S. Cosnier, C. Innocent, Y. Jouanneau, *Anal. Chem.* 66 (1994) 3198.
- [151] A.N. Reshetilov, P.V. Iliasov, H.J. Knackmuss, A.M. Boronin, *Anal. Lett.* 33 (2000) 29.
- [152] M.A. Alawi, *Fres. Z. Anal. Chem.* 313 (1982) 239.
- [153] M. Noufi, C. Yarnitzky, M. Ariel, *Anal. Chim. Acta* 234 (1990) 475.
- [154] L. Xie, C. Lu, F. Tian, *Huaxue Fence* 36 (2000) 289.
- [155] P.G. Desideri, L. Lepri, D. Heimler, in: A.J. Bard (Ed.), *Encyclopedia of the Electrochemistry of the Elements*, vol. 1, Dekker, New York, 1973, p. 104.
- [156] G.A. Sachetto, G. Favaro, P. Pastore, M. Fiorani, *Anal. Chim. Acta* 294 (1994) 251.
- [157] I. Isildak, A. Asan, *Talanta* 48 (1999) 967.
- [158] G. Högg, G. Steiner, K. Cammann, *Sensors and Actuators B* 18-19 (1994) 376.
- [159] L. Campanella, C. Colapicchioni, G. Crescentini, M.P. Sammartino, Y. Su, M. Tomassetti, *Sensors and Actuators B* 26-27 (1995) 329.
- [160] F. Zuther, K. Cammann, *Sensors and Actuators B* 18-19 (1994) 356.
- [161] I.G. Canal, J.L.F.C. Lima, M.C.B.S.M. Montenegro, R. PrezOlmos, *Analisis* 25 (1997) 32.
- [162] Y. Li, *Huaxue Shijie* 41 (2000) 214.
- [163] P. Englmaier, *J. Chromatogr.* 270 (1983) 243.

- [164] M.J. Dunphy, D.D. Goble, D.J. Smith, *Anal. Biochem.* 184 (1990) 381.
- [165] A.A. Johnson, D.G. Bursleson, *Anal. Biochem.* 236 (1996) 331.
- [166] D. Tsikas, R.H. Böger, S.M. Bode-Böger, F.M. Gutzki, J.C. Frölich, *J. Chromatogr. B.* 661 (1994) 185.
- [167] S. Kage, K. Kudo, N. Ikeda, *J. Chromatogr. B* 742 (2000) 363.
- [168] J.K. Thomsen, R.P. Cox, *J. Chromatogr.* 521 (1990) 53.
- [169] A. Tanaka, N. Nose, H. Iwasaki, *J. Chromatogr.* 235 (1982) 173.
- [170] M.I.H. Helaleh, T. Korenaga, *J. Chromatogr. B* 744 (2000) 433.
- [171] D. Grosjean, *Environ. Sci. Technol.* 24 (1990) 77.
- [172] J.P. Landers, *Clin. Chem.* 41 (1995) 495.
- [173] M.C. Barciela Alonso, R. Prego, *Anal. Chim. Acta* 416 (2000) 21.
- [174] M. Jimidar, C. Hartmann, N. Cousement, D.L. Massart, *J. Chromatogr. A.* 706 (1995) 479.
- [175] L. Cruz, L.L. Moroz, R. Gillette, J.V. Sweedler, *J. Neurochem.* 69 (1997) 110.
- [176] D. Kaniansky, I. Zelenský, A. Hybenová, F.I. Onuska, *Anal. Chem.* 66 (1994) 4258.
- [177] A.A. Okemgbo, H.H. Hill, W.F. Siems, S.G. Metcalf, *Anal. Chem.* 71 (1999) 2725.
- [178] G.M. Janini, K.C. Chan, G.M. Muschik, H.J. Issaq, *J. Chromatogr. B.* 657 (1994) 419.
- [179] K. Fukushi, N. Ishio, M. Sumida, S. Takeda, S. Wakida, K. Hiio, *Electrophoresis* 21 (2000) 2866.