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Re-Examination of the Cadmium Reduction Method and Optimisation of Conditions for the Determination of Nitrate by Flow Injection Analysis

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Abstract. The present paper re-examines the widely used flow injection method for nitrate which is based on heterogeneous reduction of nitrate with copperized cadmium followed by spectrophotometric detection of nitrite formed. The thorough investigation presented here has shown that the reduction step is very critical as far as conversion rate, possible further reduction of nitrite and long-term stability of the reductor column is concerned. The reasons for inconsistent and irreproducible results mentioned in original papers and often obtained in routine applications could be traced back to the way the reductor material had been prepared, the chemical conditions under which reduction takes place (i.e. pH and concentration of complexing agents) and the poisoning of the cadmium surface by sample constituents. The concomitant reduction of dissolved oxygen has been identified as a potential problem causing loss of pH control (due to in-situ generation of hydroxide). De-oxygenation of the carrier solution was found to be a means to overcome this problem with the additional advantages of increasing the lifetime of the reductor column and significantly reducing the concentration of cadmium ions in the waste stream. The role of particle size and column dimension as well as sample residence time within the reductor column on reduction efficiency and sample dispersion has also been investigated.

Key words: Nitrate determination; cadmium reduction method; flow injection analysis.

Nitrate is one of several states of nitrogen compounds in the geochemical nitrogen cycle, and its concentration has to be determined in a large variety of environmental samples on a routine basis. Beside ion chromatography, which over the last three decades has received acceptance as the method of choice for simultaneous determination of several anions (including nitrate), spectrophotometry is widely used for the determination of nitrate. This is partially due to the inherent instrumental simplicity of spectrophotometry, the relatively low cost of investment and operation and the fact that spectrophotometric methods for nitrate are embodied in several official standard methods (US-EPA, EN, CEN, VDI, etc.). Moreover, certain limitations with ion chromatography resulting from complex matrices, e.g. high ionic strength, presence of organic matter that can be irreversibly adsorbed on the separation column, do not exist or can be simply alleviated when spectrophotometry is used.

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A variety of spectrophotometric methods for the determination of nitrate has been described [1–3]. Many of them rely on the pre-reduction of nitrate to nitrite and determination of the latter. Here, the sum of nitrite originally present in the sample plus that formed by reduction is determined and the nitrate content calculated by difference. Reduction of nitrate can be accomplished in a different manner. It is either performed in homogeneous solution with hydrazine and copper ions as catalyst [3] or heterogeneously using metallic reductors. With respect to the latter, numerous reductants have been proposed [e.g. 4–6]. Copperized cadmium packed into microcolumns appears to be the most widely adopted method for determining nitrate in water samples, and different versions (both manual and flow procedures) have been published in the past years [e.g. 5–20].

Flow injection (FIA) determination of nitrate using copperized cadmium as the reductant is frequently used in practice and has recently received the status of an official standard method [13]. However, the application of this method in our laboratory has repeatedly led to difficulties which could always be traced back to the reduction step but were obviously not reasoned by improper use. Although we strictly adhered to the procedural details of the published methods, sensitivity, reduction efficiency, precision and long-term stability were variable, and measures to remedy the performance (also given in some original papers) were not always successful. We also realized that a procedure that worked satisfactorily on one day failed to perform comparably on another day.

Our observations were basically confirmed by correspondence with manufacturers of FIA instrumentation and in particular with working groups that routinely applied this method in environmental laboratories. The relevant literature also hints at occasional malfunction without clear evidence of the reasons [14, 16, 18, 19]. The situation vaguely reminded us of a quotation cited by Nydahl many years before when discussing the state of experience with metallic reductors for nitrate reduction: “Which kind of reductor to use and how to prepare it, as well as how to control the different conditions governing the reduction process, are matters of very different opinion. What seems to be a suitable procedure in one laboratory does not always function in another” [5]. Due to the widespread use of the method, we have initiated a thorough investigation of possible reasons for this highly unfortunate situation. A literature survey revealed that the principle procedures in the published papers are quite similar, but information regarding preparation of the cadmium material, geometry of the micro-column, flow rates of sample through the column and the chemical conditions under which the heterogeneous reduction takes place are sometimes not given at all or only insufficiently detailed to allow comparison in one or another respect.

The present paper reports on the results of an extensive study on the cadmium reduction method under FIA conditions. Critical steps in the preparation of the reductor material as well as the influence of the operational parameters after installation of the column on the performance are outlined. Particular attention was paid to identify the reasons for loss of performance with time, and measures designed to extend the lifetime are proposed. The environmental problem arising from the use of this method is also discussed.

Experimental

Chemicals and Solutions

All reagents used were of highest analytical grade. Water for preparation of solutions was from a Milli-Q purification system (>18 MOhm cm). Nitrate standards were prepared from a 1000 mg L^{-1} stock solution (obtained by dissolution of 6.071 g sodium nitrate in 1 L of water) by serial dilution with water. The buffer solution was prepared by appropriate weighing of ammonium chloride into water and adjusting the pH to the desired value by addition of ammonia solution. Alternatively to ammonium chloride the imidazole buffer solution described in the official standard method [13] was also used. For the preparation of the colour reagent, 5 g sulphanilamide and 0.5 g N-(1-naphthyl)ethylenediaminodihydrochloride were weighed into a 500 mL flask. Then 35 mL concentrated hydrochloric acid (32% w/w) was added and, after dissolution of the solid, filled up to the mark with water. This solution was stored in an amber glass bottle. Carrier and reagent solutions were degassed by membrane filtration over 0.45 µm cellulose acetate filters using water vacuum.

Cadmium raw materials[1] for filling the reductor columns were obtained from Merck (“Cadmium grob gepulvert zur Füllung von Reduktoren”, Cat. No. 102001) and Johnson Matthey (Cadmium powder, 100 mesh, Cat. No. 31420906). The Merck material was sieved in order to obtain different fractions with particle sizes in the range of approximately 0.1–0.3 mm, 0.3–0.5 mm and 0.5–0.8 mm, respectively. Various procedures for preparing the cadmium metal prior to insertion into the microcolumns were applied. The details are given under Results and Discussion. The copperized cadmium tube was from Alpkem (Clackamas, Oregon, USA) and especially prepared for in-line reduction of nitrate using their air-segmented flow analyser.

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[1] Cadmium is hazardous and requires appropriate safety considerations when handling. Discharge of cadmium waste must be in accordance with governmental regulations

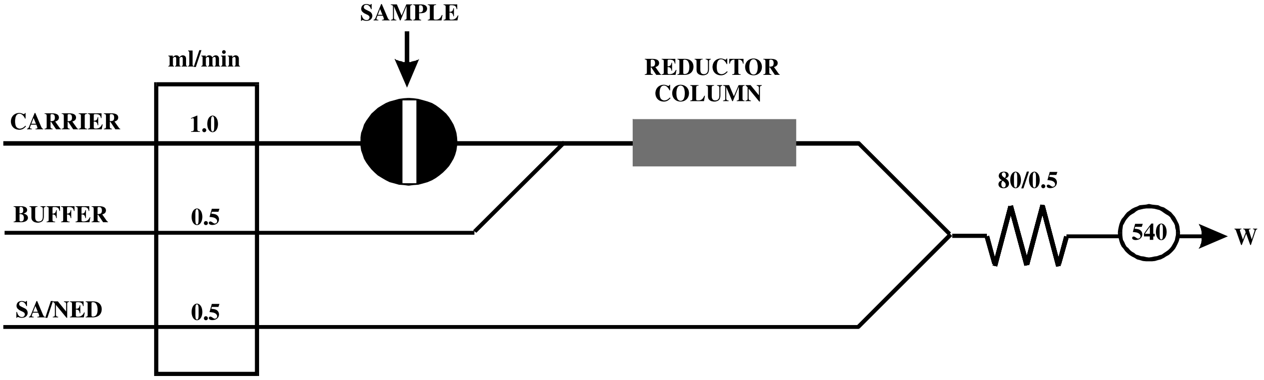

Fig. 1. General flow scheme used for investigation of heterogeneous nitrate reduction with cadmium reductor column. For further details see Experimental

Apparatus

A purpose-made flow injection system (see Fig. 1) was built comprising a multi-channel peristaltic pump (IPS-8, Ismatec, Zurich), a rotary 6-port injection valve (Latek, Heidelberg), Kel-F mixing tees and PTFE tubing (0.5 mm i.d.) of desired length. Interconnections were made using standard chromatography fittings. A Foss-Tecator spectrophotometer (Model 5032, Tecator, Höganäs) fitted with a 1-cm Hellma flow-through cuvette was used as detector. The wavelength for detection of the azo-dye formed in the reaction between nitrite and the colour reagent was set to 540 nm. The detector output was connected to a strip-chart recorder and signals were evaluated manually by peak height measurement.

A Varian SpectrAA 400 AAS instrument was used for the determination of cadmium and copper concentrations in the waste line. An acetylene-air flame was used for atomisation, and conditions were set according to the manufacturer's recommendation.

Preparation of the Reductor Columns

Reductor columns of various sizes were prepared and filled with cadmium materials that were prepared in different manners (vide infra). In addition, one commercially available ready-to-use reductor column (Foss Tecator, Part No. 50003139) was used. The geometrical dimensions of the various columns are given in Table 1. To pack the columns with the coarse material (0.1–0.8 mm particle size), the column was filled with buffer, and the individual particles were transferred into the column using tweezers. Care was taken to obtain a dense packing result (occasional soft tapping supported settlement of the granules) with a minimum dead volume and to avoid air bubble entrapment. The powder material was filled into the columns as a slurry dispensed in buffer solution using a micropipette. In order to keep the cadmium material in place, either polyethylene frits of 20 µm pore size (for the coarse material) or glass wool plugs (for the fine powder) were used.

General Procedure

In order to examine the effects of variable experimental conditions on the performance of the system, the basic flow system shown in Fig. 1 was used. The dimensions of the interconnection tubing and reaction coils were set according to those given in the standard reference method [13]. If not otherwise stated, the flow rates of the three channels were set as given in Fig. 1 compromising the residence time of the sample plug within the reductor column, analysis time and sample dilution. The reduction efficiency was

Table 1. Dimensions and particle size of the columns investigated and their effect on sample retention time. The efficiency was calculated by comparing the signals obtained for injection of NO_2^- and NO_3^- standard solutions in the concentration range of 0.1–5 mg L^{-1}, respectively

Column identification	Column dimension			Residence time [s] at 1 mL min^{-1}	Efficiency [%]
	Length [cm]	Inner diameter [mm]	Particle size [mm]		
1	6.3	4	0.2–0.5	12	98–100
2	6.3	4	2–4	15	82–100
3	5.7	4	0.2–0.5	11	96–98
4	4.7	4	0.2–0.5	9	94–96
5	3.1	4	0.2–0.5	6	70–72
6	1.5	4	0.2–0.5	3	63–65
7	0.6	4	0.2–0.5	1.2	27–29
8	9.3	2.6	0.05–0.2	1	99–100
9	7	4	0.001	0.8	99–100
10	3.1	3	0.001	0.2	98–99
11	7.6	1.2	0.2–0.5	2.5	57–59
12	72.5	1	Tube	40	23–25

Columns no. 8 and 12 are commercial ones supplied by Foss/Tecator and Alpkem, respectively.

calculated by comparing the signals obtained for standard solutions containing nitrite and nitrate of the same molar concentration.

The effect of the column dimensions on sample dispersion was investigated by injecting different volumes of nitrite standard solutions with microcolumns alternatively installed or removed from the system. In order to check whether nitrite was reduced further (the term *overreduction* is used for this in the remainder of the article), a nitrite standard solution was used as the carrier, and signals obtained with and without a reduction column in place were compared. The same signals are expected for both cases if nitrite is not reduced further.

No particular attempts were made to optimise the spectrophotometric reaction of nitrite with respect to sensitivity, since only the relative response between nitrite and nitrate under different conditions and the change of response with time was of interest. Therefore, the data given generally are presented as ratios or percentages referring to an initial value.

In order to investigate the loss of cadmium material with time, the concentration of cadmium and copper ions in the outlet of the microcolumn was determined, and the oxidation rate of the reductor material was calculated taking into account the flow rate of solution.

Results and Discussion

The reduction of nitrate using copperized cadmium followed by determination of nitrite according to one of the many variations of the Griess-Ilosvay reaction had already been proposed in the late sixties of the last century [4], and since then this method has certainly become the one most frequently used for spectrophotometric determination of nitrate [e.g. 5–20]. Alternative detection methods for nitrite following cadmium reduction of nitrate have also been proposed. They include other spectrophotometric [20], catalytic [21] and electrochemical detection schemes [22, 23].

However, there are surprisingly few papers on the mechanism of reduction and the parameters affecting the reduction process. The most comprehensive study is certainly that of Nydahl [5]. His thorough investigation of the optimum conditions for heterogeneous nitrate reduction can serve as a source of information, although it is focused more on the use of pure cadmium as reductor material. Moreover, the author worked with comparably large reductor columns and long sample residence times (followed by a batch procedure for the determination of nitrite formed) as opposed to conditions occurring in fast flow injection procedures relevant in the present investigation. Therefore it seems appropriate to recall the fundamentals of the method, to investigate the influence of the type of reductor column preparation and to examine the effects of the chemical conditions on nitrate reduction.

Fundamentals of the Method

The overall redox reaction between cadmium and nitrate leading to nitrite can be written as

$$NO_3^- + Cd + 2H^+ \rightarrow NO_2^- + Cd^{2+} + H_2O \qquad (1)$$

Accordingly, with increasing acidity the reduction of nitrate is expected to proceed faster. However, further reduction of nitrite to hydroxylamine and ammonia is possible, and the likelihood increases with decreasing pH. According to earlier studies [5, 21], the reduction of nitrate (without the risk of overreduction) occurs even at high pH (some authors have worked at pH even above 9), but the kinetics for nitrate reduction are slow, requiring long contact times between sample and reductor material. In addition, the risk of precipitation of released cadmium ions and also that of metal ions present in the sample increases with increasing pH. According to the data in the literature, a suitable pH range appears to be 6.5–8.5 compromising the risks of overreduction and precipitation.

The role of copperization of the cadmium reductor is to create a local galvanic cell at the cadmium surface that provides significantly enhanced reduction power over pure cadmium. Therefore, the reduction kinetic is enhanced and the risk of overreduction is lowered. As a result, the requirements of strict pH control in a narrow range are higher when copperized cadmium is used. On the other hand, it allows complete reduction of nitrate within a short time, which is important when fast flow injection procedures are applied. The pH range typically used in the procedures published in original papers varies over a wide range from 4.5–9 [10, 11, 13–15, 19]. Although this parameter is obviously of major importance, in several papers the pH at which reduction takes place has not been stated and can not be estimated from the preparation protocol of the reducing medium [12, 16–18, 20, 21].

Nydahl [5] has also drawn attention to the fact that dissolved oxygen is reduced by cadmium according to Eq. (2):

$$Cd + \frac{1}{2}O_2 + 2H^+ \rightarrow Cd^{2+} + H_2O \qquad (2)$$

The consumption of protons leads to a decrease in pH along the reductor columns, the magnitude of which depends on the concentration of oxygen and the buffer capacity of the solution. Nydahl investigated the pH change for different experimental conditions. For non-buffered solutions, a change in pH by more than two units was observed. In practice, nitrate reduction with copperized cadmium in FIA is very often done with ammonium chloride solution. Since the concentrations used are sometimes low, and often non-buffered solutions are used, it must be assumed that pH control during reduction is basically lost. The recently introduced official standard method [13] recommends the use of an imidazole buffer at pH 8.5 (which offers a relatively high buffer capacity), but also contains a remark stating that ammonium chloride can be used as an equivalent alternative.

We also measured the pH change in ammonium chloride solutions of various concentrations with and without pre-adjustment of the pH upon passage through the reductor column. The results we obtained basically confirmed the data of Nydahl in that an ammonium chloride concentration $>0.5\,\mathrm{mol\,L^{-1}}$ adjusted to pH 8–9 is required to keep the pH in a narrow range. When using the imidazole buffer according to ref. 13, the buffer capacity is sufficient to prevent a measurable pH change. It is noteworthy that for the analysis of acidic samples and samples with high buffer capacities, the chemical conditions need to be appropriately adjusted.

We also investigated the chemical effects on reduction efficiency, likelihood of overreduction and on the stability of response with time with a copperized cadmium column under FIA conditions. Although not all results can be presented here, we can conclude that (i) the lowest risk of overreduction is given at pH above 8.0, (ii) freshly prepared columns (at any pH) tend to show a higher risk of overreduction compared to columns that have been used for several hours; (ii) overreduction is significantly enhanced if copper ions are added to the carrier or buffer (as recommended in some papers to stabilise the performance [14]), (iii) with increasing sample residence time, the risk of overreduction also increases, (iv) under none of the conditions that ensure complete reduction of nitrate to nitrite could overreduction be completely eliminated. The lowest rates observed were 3–5% at pH 8.5.

At pH values above 8.5, precipitation of cadmium and copper hydroxide may occur, causing reduced reduction efficiency and irreproducible results. In the presence of complexing ions (e.g. ammonia for copper, imidazole for copper and cadmium), precipitation is depressed (the degree depending on the relative concentrations of the ions involved) and performance is restored. The addition of EDTA proposed in previously published papers [10–12, 14, 21] (without evidencing the necessity or its effect) certainly has the same function.

Preparation of Cadmium Materials

The typical procedure for preparing the cadmium reductor material involves briefly treating the raw metal with hydrochloric acid to remove surface layers, washing off the acid with water, and treatment with copper sulphate to obtain the copperized cadmium. Finally the material is washed with buffer solution and stored wet until it is filled into the column. However, the concentrations of solutions and time of contact used by various workers differ significantly (if given at all). Since the preparation of the reductor material was thought to be one possible reason for the observed inconsistencies in the performance of the method, we investigated the effect of treatment conditions on the reduction behaviour. Raw and acid-treated cadmium materials were used with and without copperization, and the copperization process was varied in terms of copper concentration (in relation to given amount of cadmium) and coating time.

The specific surface area of the cadmium material was also considered important since, when completely deposited, the same absolute amount of copper leads to a different thickness of the copper layer when small or coarse cadmium particles are used. The two extremes of the various copperization procedures tested, which led to thin and thick copper coatings, were a brief treatment with low concentration copper solution (approx. 4 g sieved Cd granules of 0.1–0.3 mm in diameter treated with 10 mL 0.05% copper sulphate solution for 1 min) and repeated treatment of the granules with fresh copper solution until saturation, respectively. Saturation was indicated by the formation of copper sludge, i.e. an excess of metallic copper filaments that do not adhere to the cadmium surface [10].

Visual observation of the ongoing coating process and the materials finally obtained revealed a colour change depending on the thickness of the layer and also on the composition of the solvent in contact with the cadmium. The metallic shine of acid-treated cadmium immediately disappears when copper solution is added. The cadmium surface turns light grey in copper sulphate solution and turns almost black when it is washed with ammonia buffer. Thick layers (conditions of saturation) sometimes went reddish in contact with buffer.

Due to the reduction of oxygen by copperized cadmium, we measured the associated increase in pH and found that values up to pH 10.5 were obtained in the bulk of the solution. Considering that the reduction takes place at the immediate surface, much higher local pH values can be reached. This may result in precipitation of copper and cadmium hydroxides, which can affect the morphology of the copper deposit and consequently also the reduction efficiency. Therefore, a buffered ($0.5\,\mathrm{mol\,L^{-1}}$ ammonium chloride, pH 8) copper sulphate solution was used for copperization in one experiment, while the same buffer composition was used for washing and storing the cadmium material. The effect of differently prepared cadmium materials on the performance of the reductor column was investigated with respect to reduction efficiency and lifetime. The results are included in the respective chapters.

Table 2. Effect of preparation of Cd granules on reduction efficiency (column no. 1 was used for this investigation). The efficiency was calculated by comparing the signals obtained for injection of NO_2^- and NO_3^- standard solutions in the concentration range of 0.1–$5\,\mathrm{mg\,L^{-1}}$, respectively

Column preparation	Efficiency [%]
1. Raw Cd particles (metallic shine) untreated	35–37
2. Raw Cd treated with HCl (dark grey)	27–30
3. Column no. 1 (fine particles) covered with thin layer Cu	97–100
4. Column no. 1 (fine particles) covered with medium Cu layer	97–100
5.Column no. 1 (fine particles) plated until saturation (formation of Cu sludge)	97-100

Efficiency of Nitrate Reduction

According to the principle of operation, the literature data and our own experience with this method, and apart from the chemical conditions (vide supra), the reduction efficiency for nitrate is linked to the geometry of the reductor column, the size of the cadmium particles, the way of preparing the cadmium material and the contact time between nitrate and cadmium, which in turn for the given geometrical dimensions depends on the sample flow rate through the microcolumn.

In order to investigate the effect of cadmium material preparation, a microcolumn of 6.3 cm length and 4 mm i.d., $1.2\,\mathrm{mol\,L^{-1}}$ ammonium chloride buffer and a flow rate through the column of $1.5\,\mathrm{mL\,min^{-1}}$ were used. The differently prepared cadmium granules (see previous section) were filled into the column, and after several minutes of continuous pumping the reduction efficiency for nitrate was determined at various concentration levels. It is worth noting that in most instances, it took a considerable amount of time before a stable response was obtained. This has also been observed in previous studies, and measures proposed to overcome this unfavourable behaviour (without an explanation of what the reasons could be) include injection of acid and activation with a high concentration of nitrate [13, 14, 16, 18, 19, 26, 27]. When using the copperized cadmium that was always in contact with buffer (vide supra), the response was stable from the very beginning. It is therefore assumed that the high local pH during the preparation of the reductor material causes precipitation of cadmium and/or copper hydroxide at the surface of the granules which are dissolved only slowly later on. This is confirmed by the observation that consistent and stable results are obtained faster when a high buffer concentration is used and also when the column is briefly treated with diluted hydrochloric acid in between.

The results are summarised in Table 2. They show that (after activation and stabilisation) the reduction efficiency for nitrate was close to 100% for all copperized materials, i.e. irrespective of the treatment procedure, whereas the raw materials gave only about 30–35% efficiency under same experimental conditions. The constancy of reduction efficiency was tested over a period of several hours by repetitive injections of more than 100 nitrate standard solutions within the typical working range. Every 20 minutes the carrier solution was replaced with a high nitrate standard solution for about 2–3 min ($200\,\mathrm{mg\,L^{-1}}$ $NO_3^- - N$) to simulate extremely high analyte loading conditions. No significant change in performance of the micro-columns occurred.

It is worth mentioning, however, that after few hours of continuous operation, the material with a thick copper layer turned reddish at the inlet, and fine solids were accumulated at the outlet. In one case, the fine particles were carefully removed from the column, dissolved in acid and analysed for copper and cadmium by AAS, since we supposed this most likely to be a copper precipitate. This was indeed confirmed and can easily be explained by mechanical spoil of the reasonably thick copper filaments attached to the cadmium granules [18]. Another reason for the release of the metallic copper layer is the dissolution of the cadmium according to Eqs. (1) and (2). This was in fact experimentally verified by studying the release of cadmium ions from the column and correlating this to the formation of the copper precipitate.

In the long run, i.e. when using microcolumns daily over periods of weeks and months with interruptions only during night time and weekends, the stability of response was generally maintained as long as only standard solutions were measured, if the column was stored wet in buffer solution and air bubble entrapment was avoided. Reactivation of the column by acid injection was sometimes required. Possibly a pH increase within the column occurs even in buffered solution due to diffusional transport of dissolved oxygen to the surface and its reduction. Storage under nitrogen could be a means to overcome this effect.

Effect of Column Dimension on Reduction Efficiency

After having optimised the chemical conditions, we also investigated the influence of physical parameters on the reduction efficiency. To this end, we prepared various microcolumns differing in length and inner diameter (and hence in the residence time of the sample plug passing through the column) as well as in the size of the cadmium particles. For given column dimensions, the latter has an effect on the void volume and thus on the residence time and on diffusional path length the nitrate ions have to move along in order to reach the cadmium surface where reduction takes place. The results of this investigation are condensed in Table 1. The residence times given were experimentally obtained by injection of a strongly coloured solution and measuring the time lapse between the point of entering the column and reaching the outlet frit.

Without discussing all the data of Table 1 in detail, some obvious results may be mentioned: (i) for a given particle size, the reduction efficiency decreases with decreasing length of the column (compare data for column 1 and 3–7); (ii) large particles are unfavourable even when large columns are used (compare column 1 and 2); (iii) even with short columns, high reduction efficiency can be obtained when fine cadmium particles are used (column 10 and 11); (iv) despite the long sample residence time, the open tube exhibits low reduction efficiency. This is probably due to the long diffusional path length; (v) the ready-to-use commercial microcolumn (no. 8) offers high reduction efficiency as a consequence of compromising size and column dimension.

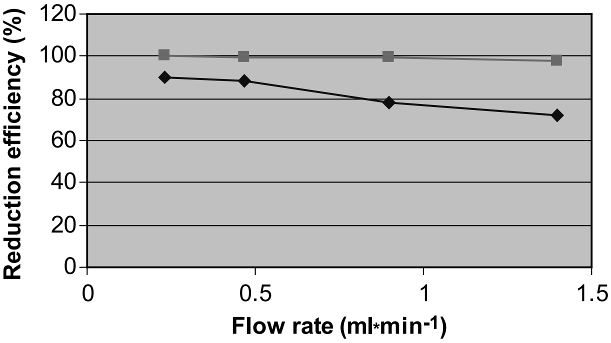

Fig. 2. Effect of flow rate on reduction efficiency as obtained for column no. 1 (■) and no. 5 (■). For column identification see Table 1. The efficiency was calculated by comparing the signals obtained for injection of NO_2^- and NO_3^- standard solutions in the concentration range of $0.1-5\,\mathrm{mg\,L^{-1}}$, respectively

The effect of the flow rate on column efficiency was exemplarily investigated using columns no 1 and 5. The results are shown in Fig. 2. In the flow rate range of 0.2–1.4 $\mathrm{mL\,min^{-1}}$, no significant deviation from 100% efficiency is observed for the large column, but for the shorter column efficiency decreases with increasing flow rate, which is probably a result of the too short residence time. Similar observations were made with other columns of smaller dimensions that do not exhibit 100% reduction efficiency.

Influence of Column Dimensions on Sample Dispersion

Sensitivity in flow injection analysis is ultimately related to sample dispersion. The parameters affecting dispersion are injection volume, flow rates (absolute and ratios between the streams), and the length and inner diameter of the tubing. Microcolumns installed into the manifold due to inherent dead volumes can act as mixing chambers and significantly contribute to dispersion. The influence of the geometry of various columns on dispersion was therefore investigated. Measurements were made with two different injection volumes, and the minimum volume required to reach a steady state signal was also investigated. The results given in Table 3 show that the least contribution to dispersion is given by columns no 8, 10 and 11. Since only no 8 and 10 exhibit 100% reduction efficiency (see Table 1), they can be regarded as the most suitable ones. Of course the dispersion effect can be

Table 3. Influence of column dimensions on sample dispersion (for column identification see Table 1). The nitrate concentration in this particular experiment was 1 mg L^{-1}

Column identification	Dispersion contribution of column*		Minimum sample volume to obtain no dispersion(μL)
	10 μL injection	200 μL injection	
1	7.7	4.5	650
2	8.4	5.3	900
8	4.7	3.2	450
9	8.2	5.6	850
10	5.1	3.8	480
11	5.2	3.5	450
12	10.4	6	1200

* Dispersion is defined as the ratio of signal heights obtained for continuous aspiration and injection of a standard solution of given concentration.

completely avoided using sufficiently large injection volumes. However, apart from higher sample consumption this has adverse effects on sampling frequency and may load the column with contaminating sample constituents.

Performance Characteristics of the Method

The foregoing chapters make clear that the method's best performance characteristics are obtained when the reduction efficiency is close to 100% and the contribution to sample dispersion of the microcolumn is at a minimum. Hence the commercial column from Foss Tecator (no. 8) and the short column filled with cadmium powder (no. 10) are optimal. The latter column was sometimes difficult to apply because of considerable backpressure. The method was calibrated with columns no. 1 and no. 8 in the concentration range of 0.01–1 mg L^{-1}. When using a 400 μL injection volume, sensitivity for the two columns was 1.04 and 1.28 L mg^{-1}, which is very close to that stated by manufacturers [26, 27] and considerably better than the requirement set out in the official standard method [13] . The detection limit of the method (calculated as 3σ of the blank) was 3 μg L^{-1}. The typical precision of repetitive injections was always below 5% relative standard deviation over the entire concentration range.

Lifetime of the Reductor Column

The main reason for applying flow injection methods in routine analysis is being able to perform automated sample processing of large numbers of samples with low reagent consumption and minimum maintenance. With respect to the nitrate reduction method, the most critical part is surely the reductor column. According to Eqs. (1) and (2) cadmium is dissolved by oxidation processes, and this ultimately leads to loss of the reductor material with time.

Concerning the reduction of nitrate only, a simple stoechiometric calculation shows that about 10^{-2} mol nitrate can be reduced with a typical amount of cadmium (i.e. 1–5 g). Assuming an injection volume of 100 μL and a mean nitrate concentration of 1 mg L^{-1}, this corresponds mathematically to about 10 million injections. A similar calculation for the consumption of cadmium due to the reduction of dissolved oxygen evidences that this is by far the most predominant route of cadmium loss. It must be considered that oxygen is present in carrier and buffer solutions and is hence continuously provided whether samples are injected or not. It therefore appeared useful to deoxygenate carrier and buffer streams.

The effect of deoxygenation on cadmium release was followed directly by the determination of cadmium ions in the waste line. It was found that at a combined flow rate of buffer and carrier of 3 mL min^{-1} (no nitrate present), the cadmium ion concentration decreased from about 150–220 mg L^{-1} to about 1–4 mg L^{-1} Cd^{2+} or, in terms of absolute oxidation rate, from about 200 ng min^{-1} to 2 ng min^{-1} Cd. If the carrier was replaced with a deoxygenated nitrate standard of 1 mg L^{-1}, no significant increase in the release of cadmium ions was observed.

The loss of cadmium from the column is unfavourable for several reasons. First of all there is the environmental aspect. Although the use of cadmium is problematic altogether, continuous production of liquid waste with high cadmium content is so to an even greater extent. The almost 100-fold lower cadmium concentration under deoxygenated conditions is an obvious advantage. A second problem is that the loss of cadmium from the column causes dead volumes that have a negative influence on sample dispersion. Finally, since small cadmium particles are dissolved first, the overall specific surface area available for reduction decreases quickly with adverse effects on reduction efficiency [19].

After having identified the aforementioned problems, we worked with deoxygenated solutions and strict pH control for a considerable length time. The stability of response (during initialisation and also long term) could be significantly improved, and many of the shortcomings which reasoned this investigation (see Introduction) did not occur any longer.

However, there are also other reasons for loss of performance with time that are related to the composition of the samples, i.e. adsorption of high molecular weight organic matter on the surface of the cadmium material, poisoning by deposition of metals (present in the sample in ionic form) more noble than cadmium, air bubble entrapment due to incidental injection of air and clogging of the frits due to particles.

Deactivation by organic matter and clogging by particles can simply be prevented by in-line dialytic sample treatment [13, 28–30]. In our experience this is a very robust method that permits interference-free analysis of coloured and turbid samples. Although dialysis is generally connected with a considerable loss of sensitivity, this can partially be remedied by the recently proposed stopped-flow approach [31]. Air bubble entrapment within the microcolumns is a serious problem, since reduction efficiency is drastically reduced and sample dispersion enhanced. Attempts to remove air bubbles without disconnecting the microcolumns generally failed. It is worth mentioning that air bubbles do not stick in columns filled with fine cadmium powder material or in the cadmium tube. However, a problem with the former is the high backpressure when columns of larger dimensions are applied and with the latter that reduction efficiency is low.

Attempts of Regeneration of Microcolumns

The loss of reduction efficiency with time has been mentioned in several original papers [14, 16, 19] and has also been confirmed in discussions with representatives of FIA equipment manufacturers. A general means proposed to reactivate the column is successive treatment with diluted hydrochloric acid and copper sulphate solution (simulating basically the procedure of initially coating the cadmium material). In our experience this treatment indeed generally led to temporary restoration of the performance. However, the column lifetime after this treatment was considerably decreased (sometimes high reduction efficiency was only maintained for the first 1–2 hours of operation) and after repeated treatment the performance ceased even more quickly.

Visual inspection of the column revealed that a prolonged (or repeated) treatment with copper sulphate caused the cadmium granules close to the entrance to become reddish (typical copper colour) with time. Hence it is believed that the copper deposit had become too thick to enable nitrate reduction by the copper/cadmium couple. Therefore, we can not recommend in-line treatment procedures as a reliable means of durable reactivation.

Another reason for deactivation is the deposition of high molecular weight compounds (e.g. humic and fulvic acids, proteins, oil and grease) on the cadmium surface. Attempts to remove these layers by treating with surface-active compounds and/or addition of water-miscible organic solvents only occasionally (and for a short time) led to some improvement, but generally this could not remedy the performance satisfactorily.

Conclusions

The present paper re-examines the performance of the well-established and frequently employed cadmium reduction method for nitrate determination because of severe problems and occasional irregularities that have been repeatedly encountered in practice. Generally, the main problem was to maintain high reduction efficiency and long-term response stability. Our experimental study has revealed that the heterogeneous reaction is likely to be affected by the kind of cadmium material used (size, pre-treatment, storage conditions), the residence time of the sample within the reductor column (which in turn depends on geometrical dimensions and flow rate) and the chemical composition of the medium in which the reduction takes place. With respect to the latter, the results can be summarized as follows: (i) sample must be buffered appropriately to pH 8–8.5; (ii) the presence of complexing agents is mandatory to prevent hydroxide precipitation; (iii) if ammonium chloride is used as a buffer, the concentration should be at least $0.5\,\mathrm{mol\,L^{-1}}$.

The lifetime of the column is ultimately related to the amount of potential oxidants for cadmium passing through. Dissolution of cadmium by dissolved oxygen is the dominant reaction. Deoxygenation of the carrier and buffer streams is thus recommended, having a beneficial side-effect of considerably decreased cadmium ion concentration in the waste line. With respect to sensitivity of the FIA method, the contribution to sample dispersion upon passage of the sample plug through the microcolumn has been shown to strongly depend on the overall size of the reductor column but also on the size of the cadmium granules and the compactness of the packing. Columns filled with cadmium powder and those of small inner diameters and fine particles gave the best results.

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