Environ Sci Pollut Res (2015) 22:9575–9588
DOI 10.1007/s11356-015-4522-7

REVIEW ARTICLE

Ion-exchange method in the collection of nitrate from freshwater ecosystems for nitrogen and oxygen isotope analysis: a review

Wen-Bing Li[1,2] · **Yao-Bin Song**[1] · **Hong-Ke Xu**[1] · **Ling-Yun Chen**[1] · **Wen-Hong Dai**[1] · **Ming Dong**[1,2]

Received: 17 December 2014 / Accepted: 9 April 2015 /

Abstract Nitrate (NO_3^-) contamination of freshwater is considered one of the most prevalent global environmental problems. Dual stable isotopic compositions ($\delta^{15}N$ and $\delta^{18}O$) of NO_3^- can provide helpful information and have been well documented as being a powerful tool to track the source of NO_3^- in freshwater ecosystems. The ion-exchange method is a reliable and precise technique for measuring the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- and has been widely employed to collect NO_3^- from freshwater ecosystems. This review summarizes and presents the principles, affecting factors and corresponding significant improvements of the ion-exchange method. Finally, potential improvements and perspectives for the applicability of this method are also discussed, as are suggestions for further research and development drawn from the overall conclusions.

Keywords Freshwater · Ion exchange · Isotopes · Nitrate · Ecology · Pretreatment

Abbreviations

WHO	World Health Organization
IRMS	Isotope ratio mass spectrometer
USGS	United States Geological Survey
EA-IRMS	Elemental analyzer isotope ratio mass spectrometry
IAEA	International atomic energy agency
H/D	Height to diameter

Introduction

Nitrate (NO_3^-) contamination of freshwater (such as surface and groundwater) is considered one of the most prevalent global environmental problems and is responsible for freshwater ecosystem deterioration on a worldwide scale (Ding et al. 2014; Mallin et al. 2006; Rabalais 2002; Smith 2003; Xue et al. 2010). In many developed regions of the world, nitrate levels in surface and groundwater have risen substantially because of crop and human N fixation; the latter includes synthetic fertilizer production for agriculture and the incidental generation of N-bearing compounds via the combustion of fossil fuels (Boumans et al. 2004; Burgin and Hamilton 2007; Liu et al. 2013; Michalski et al. 2004; Thorburn et al. 2003; Xue et al. 2010). High levels of nitrate can threaten the health of humans, livestock, and freshwater ecosystems. For instance, ingested nitrates can cause methemoglobinemia (namely the commonly known cyanosis or blue baby syndrome) in infants by the conversion of nitrate to nitrites in their guts causing the decrease of the oxygen-carrying capacity of hemoglobin (Camargo and Alonso 2006; Camargo et al. 2005; Fukada et al. 2003; Liu et al. 2006). In addition, ingestion of nitrate-contaminated water may increase the likelihood of stomach cancer because of the formation of nitrosamines, which are one of the most notorious carcinogens in mammals (Camargo et al. 2005; Panno et al. 2006; Xing and Liu 2011). As a result, a limit of 10 mg NO_3^-–N L^{-1} for drinking water has been set by the World Health Organization (WHO) (Xue et al. 2009). Furthermore, elevated concentrations of nitrate

Responsible editor: Philippe Garrigues

✉ Ming Dong
dongmingchina@126.com

[1] Key Laboratory of Hangzhou City for Ecosystem Protection and Restoration, College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 310036, China

[2] State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

can stimulate eutrophication and hypoxia in freshwater ecosystems adversely affecting aquatic species (Camargo and Alonso 2006; Lenihan and Peterson 1998; Liu et al. 2013). In response to these problems, the identification of nitrate sources and implementation of appropriate actions are imperative. However, understanding the sources of nitrate in freshwater ecosystems is a challenging task because nitrate can be derived from multiple sources, such as synthetic fertilizer (Bateman and Kelly 2007; Di and Cameron 2002), manure (Choi et al. 2003, 2007; Wassenaar 1995), sewage (Aravena et al. 1993; Li et al. 2007; Widory et al. 2005), atmospheric deposition (Pardo et al. 2004; Russell et al. 1998; Williard et al. 2001; Zhang et al. 2008), and microbial nitrification processes (Mayer et al. 2001, 2002). Traditionally, identification of nitrate sources can be achieved by investigation of the nitrate concentration in conjunction with discharge data, land use, and the hydrological character of contaminated regions (Chang et al. 2002; Xing and Liu 2011). However, these do not supply accurate and detailed information about nitrate sources. Because of the distinct isotopic characteristics of nitrogen and oxygen in the main sources of nitrate, stable isotopic compositions of nitrate can provide helpful information and are considered a powerful tool to track the source of nitrate in freshwater ecosystems (Deutsch et al. 2006; Li et al. 2010; Xue et al. 2010). Kohl et al. (1971) first introduced nitrogen isotopes to estimate the contribution of fertilizer to the nitrate in the Sangamon River (Illinois, USA), which laid the groundwork for the application of stable isotopes to distinguish the sources of nitrate contamination in freshwater ecosystems. During the last two decades, the dual stable isotope analysis technique, which combines the analysis of δ^{15}N–NO_3^- and δ^{18}O–NO_3^-, has drawn substantial attention and has been widely used to track sources of nitrate in freshwater ecosystems (Ahmed et al. 2012a, b; Minet et al. 2011). Currently, two principal analytical techniques are used for the determination of δ^{15}N–NO_3^- and δ^{18}O–NO_3^-: the denitrification method (Casciotti et al. 2002; McIlvin and Altabet 2005; Sigman et al. 2001) and the ion-exchange method (Chang et al. 1999, 2002; Minet et al. 2011; Silva et al. 2000). The characteristics of the different methods are presented in Table 1. The denitrification method, which allows the determination of both δ^{15}N and δ^{18}O in nitrous oxide (N_2O) resulted from nitrate by chemical (McIlvin and Altabet 2005) or by bacterial method (Casciotti et al. 2002; Rock and Ellert 2007; Sigman et al. 2001), have two disadvantages: (1) The nitrite (NO_2^-) presented in water samples would overestimate or underestimate the isotopic composition and (2) the results require mathematical treatments to correct for oxygen exchange and fractionation (Minet et al. 2011; Xue et al. 2009). The ion-exchange method, which allows the determination of both δ^{15}N and δ^{18}O simultaneously in silver or potassium nitrate by an extraction and conversion procedure that involves physical and chemical reactions, has several advantages: (1) the ability to store samples on columns in the field and thereby not need to ship water samples; (2) the ability to preserve samples without hazardous chemicals; (3) the lower start-up cost as compared to the bacterial method; and (4) the lack of the need to continuously propagate the bacteria for the denitrification method (Ahmed et al. 2012b; Silva et al. 2000). For these reasons, the ion-exchange method has been successfully used to track nitrate transformations and sources in freshwater ecosystems such as those in surface water (Battaglin et al. 2001; Chang et al. 1999; Chen et al. 2014; Deutsch et al. 2006; Fukada et al. 2003; Lee et al. 2008; Li et al. 2010; Liang et al. 2013; Liu et al. 2006; Minet et al. 2011; Panno et al. 2006; Xue et al. 2010; Zhang et al. 2014) and in groundwater (Aravena and Robertson 1998; Fukada et al. 2003; Liu et al. 2014; Yuan et al. 2012; Zhang et al. 2014) (Table 2). However, the ion-exchange method designed by Chang et al. (1999) and Silva et al. (2000) still has some disadvantages: It is labor-intensive, time-consuming, and expensive and has thus been modified and improved accordingly.

Table 1 Characteristics of different methods for the determination of δ^{15}N–NO_3^- and δ^{18}O–NO_3^-

Criterion	Method		
	Ion exchange	Denitrification	
		Bacterial	Chemical
Applicable objects	Freshwater	Freshwater and seawater	Freshwater and seawater
Sample volume needed	High	Low	Low
Ease of sample handling	No	No	Yes
Risk of procedure	Low	Low	High
Time needed	High	High	Low
Accuracy and sensitivity	High	Low	Low
Cost	High	Low	Low

In the present article, the principles of the ion-exchange method are first introduced. Factors affecting the ion-exchange method and the corresponding significant improvements are reviewed, and the best procedure now available is concluded. Finally, potential improvements and perspectives for the applicability of this method are also discussed, with suggestions for further research and development drawn from the overall conclusions.

Principles of the ion-exchange method

The ion-exchange method, which can be used to extract nitrate from freshwater for the simultaneous determination of δ^{15}N

Table 2 Case studies of the use of ion-exchange method for nitrogen and oxygen isotope analysis of nitrate from freshwater ecosystems

No.	Sample type	Sampling site	Sample number	Range of stable isotope values				Reference
				δ^{15}N–NO_3^- (‰)		δ^{18}O–NO_3^- (‰)		
				Min	Max	Min	Max	
1	Surface water	Manure Action Plan, Belgium	42	4.10	21.90	3.20	22.70	Xue et al. 2010
2	Surface water groundwater	River Elbe basin, Germany	13	8.85	20.60	8.02	16.78	Fukada et al. 2003
3	Surface water groundwater	Guiyang, China	32	−2.70	15.40	2.80	27.00	Liu et al. 2006
4	Surface water	Mississippi River, Illinois, USA	27	4.80	16.40	6.60	13.90	Panno et al. 2006
5	Surface water	Mecklenburg-Vorpommern, Germany	10	7.20	15.00	1.80	9.10	Deutsch et al. 2006
6	Surface water	Changjiang River, China	38	−7.30	12.90	2.40	11.20	Li et al. 2010
7	Surface water and snow	Loch Vale, Colorado, USA	9	−1.80	6.00	7.30	66.10	Chang et al. 1999
8	Surface water	Mississippi River, USA	42	2.80	12.40	3.00	24.00	Battaglin et al. 2001
9	Surface water	Han River, Republic of Korea	24	4.27	11.44	0.22	7.36	Lee et al. 2008
10	Surface water	Illinois River Basin, USA	131	3.90	22.50	0.60	61.00	Panno et al. 2008
11	Surface water groundwater	North China Plain, China	60	−2.50	20.00	4.00	12.40	Zhang et al. 2014
12	Surface water	East Tiaoxi River, China	21	1.40	12.40	3.70	9.00	Liang et al. 2013
13	Surface water	Taihu Lake water system, China	17	3.32	10.38	3.30	21.50	Chen et al. 2014
14	Groundwater	Southern Ontario, Canada	34	14.60	38.80	3.50	16.60	Aravena and Robertson 1998
15	Groundwater	North China Plain, China	12	4.50	15.40	−8.12	4.32	Yuan et al. 2012
16	Groundwater	Beijing area, China	18	4.78	16.96	3.13	21.81	Liu et al. 2014

and δ^{18}O, was first designed by Chang et al. (1999) and Silva et al. (2000) and has been modified accordingly during the past decade (Ahmed et al. 2012b; Chen et al. 2010; Fukada et al. 2003; Huber et al. 2012; Minet et al. 2011; Xing and Liu 2011). As shown in Fig. 1, this method is mainly composed of six steps: sampling and pretreatment, extraction, elution, neutralization, removal of interfering ions, and drying and stable isotope analysis. Since anion-exchange resin with chloride form presents relatively high affinity for nitrate, nitrate can be extracted by passing the water samples through columns prefilled with anion-exchange resin. In this way, nitrate is extracted from water and retained onto resins. After the extraction step, the NO_3^- can be eluted from the anion-exchange resin by dripping of chloride solutions, such as hydrochloric acid (HCl) and potassium chloride (KCl) solutions. With this approach, the NO_3^- can be recovered from the anion-exchange resin (Silva et al. 2000). Nitric acid (HNO_3) and HCl coexist in the nitrate-bearing eluant from the anion-exchange resin column (Eq. 1) (Ahmed et al. 2012a, b). Because HNO_3 is volatile and isotopic fractionation will occur under low-pH conditions, it must be neutralized as soon as possible before the drying process (Ahmed et al. 2012b; Böhlke et al. 2003; Silva et al. 2000). To prevent the loss of nitrate, the eluant can be neutralized by silver oxide (Ag_2O) (Eq. 2) or combination of Ag_2O and potassium hydroxide, and the nitrate was presented as $AgNO_3$ or KNO_3, respectively. To obtain solid $AgNO_3$ salts, the solution obtained after neutralization can be freeze-dried or oven-dried (Fukada et al. 2003; Silva et al. 2000; Xue et al. 2009). Freeze-drying, which can be achieved by putting solutions overnight in a freezer or in liquid nitrogen as soon as possible, is the usual choice for the $AgNO_3$-drying process. To obtain solid KNO_3 salts, neutralized solution consisting of KNO_3 and KCl was evaporated till complete dryness on a hot plate. The dried salt mix was mixed with activated graphite and finally ground (Ahmed et al. 2012a, b; Aly et al. 2010). In this way, the nitrate is extracted from the water and converted into solid $AgNO_3$ or KNO_3 salts, which are subsequently processed for stable isotope analysis by combustion or pyrolyzation method.

$$R^+ + NO_3^- + 2HCl \rightarrow HNO_3 + HCl + RCl \qquad (1)$$

$$HCl + HNO_3 + Ag_2O \rightarrow AgCl + AgNO_3 + H_2O \qquad (2)$$

As to the combustion method for nitrogen isotopic analysis, $AgNO_3$ is transferred to silver capsules and then combusted in a combustion reactor, which is maintained at a high temperature (Li et al. 2010). The resulting nitrogen gas (N_2) produced in the oxidation reactor is introduced into an isotope ratio mass spectrometer (IRMS) for δ^{15}N analysis (Fukada et al. 2003; Li et al. 2010; Xing and Liu 2011). For

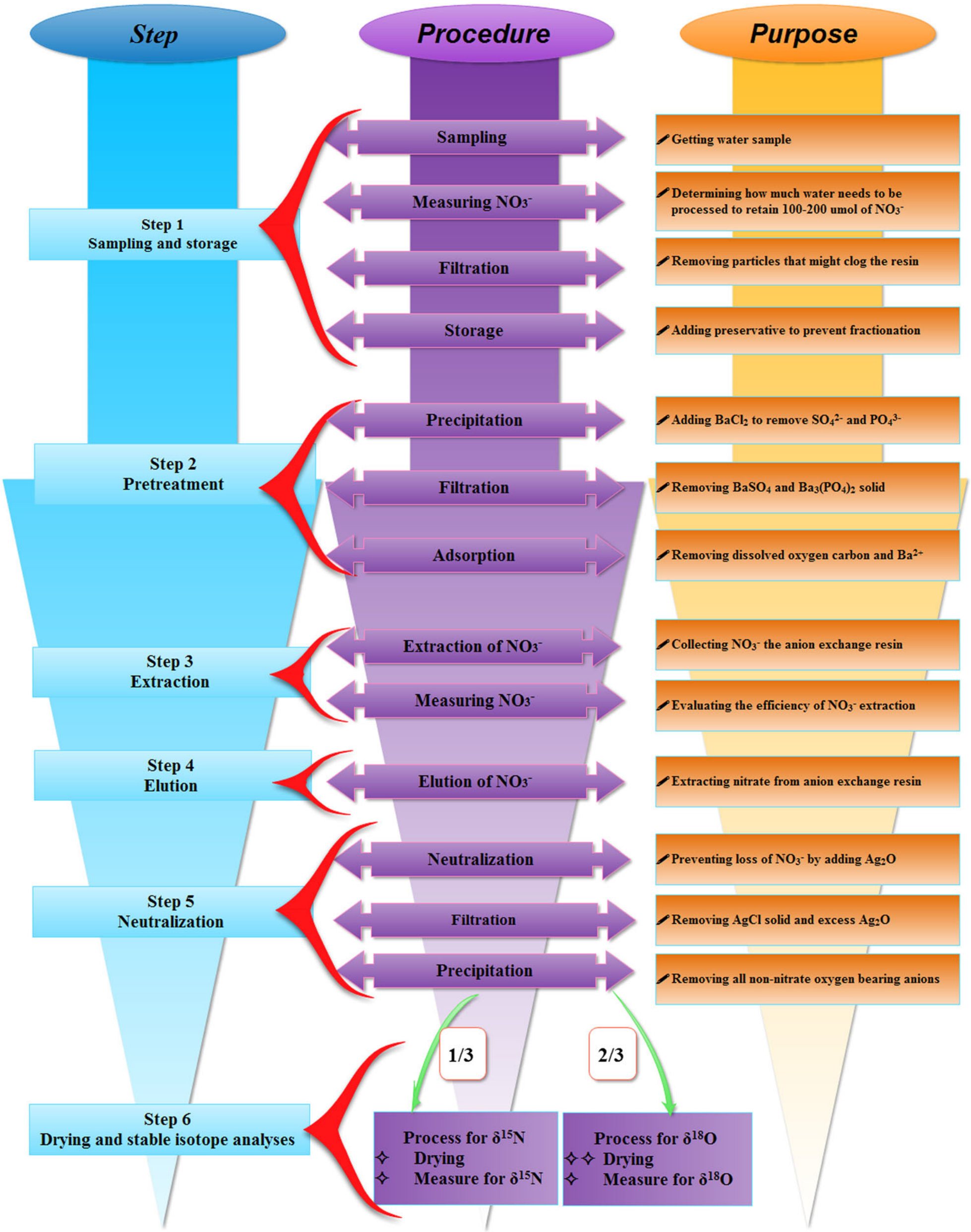

Fig. 1 Flow diagram depicting the procedure for the ion-exchange method (adapted from Chang et al. 1999)

the prepared solid KNO_3 samples, a modified catalyst-free technique was used by Aly et al. (2010), who used activated graphite as the reducing agent. The prepared KNO_3 samples were mixed with activated graphite, loaded onto a precombusted pyrex glass tubes, combusted at 550 °C for 30 min, and cooled for 2 h. In this way, the KNO_3 samples were completely decomposed and the liberated N_2 gas was available for nitrogen isotopic analysis by IRMS. As to the combustion method for oxygen isotopic analysis, $AgNO_3$ is combusted with silver cyanide (AgCN) or graphite and converted into CO_2 according to Eq. 3 and Eq. 4; the produced CO_2 is subsequently cryogenically separated from N_2 and analyzed for $\delta^{18}O$ by IRMS (Ahmed et al. 2012b; Fukada et al. 2003; Silva et al. 2000; Wassenaar 1995). Compared to AgCN, graphite is a better carbon source because it does not necessitate the disposal of hazardous chemicals. For the prepared solid KNO_3 samples, a modified catalyst-free technique was used by Ahmed et al. (2012b). The dried solid KNO_3 salt is firstly mixed with activated graphite, and then, the mixture is put into a 20 cm length and 6 mm (optical density (OD)) Pyrex glass tubes which need to be precombusted. After this step, the combustion tubes are evacuated, torch-sealed, combusted, and cooled (Ahmed et al. 2012b). In this way, the KNO_3 samples were completely decomposed and the liberated CO_2 gas was available for oxygen isotopic analysis by IRMS.

With the development of analysis technique, determination of $\delta^{15}N$ and $\delta^{18}O$ values simultaneously can also be achieved by pyrolyzation method which uses thermal decomposition without adding oxygen (Deutsch et al. 2006; Kornexl et al. 1999; Li et al. 2010; Minet et al. 2011; Xue et al. 2009). The prepared $AgNO_3$ samples are pyrolyzed in a molybdenum-lined, aluminum oxide reduction tube (filled with glassy carbon and topped with a glassy carbon crucible) which is maintained at a high temperature (1400 °C), and the produced N_2 and CO can be separated from by a 1 m gas chromatography (GC) column and analyzed via IRMS for $\delta^{15}N$ and $\delta^{18}O$ (Xue et al. 2009). In addition, oxygen stable analysis can be also achieved individually although the N_2 produced during pyrolysis can trail into the CO sample peak, which makes analyses of $\delta^{18}O$ in nitrogen-containing materials, such as nitrates and caffeine, difficult. To eliminate the N_2 interference in the CO sample peak, two methods can be adopted. First, CO and the undesirable N_2 produced by pyrolysis can be separated by a GC column packed with 5-Å molecular sieves (Li et al. 2010; Minet et al. 2011). In addition, an independent CO-trapping technique can be used and the interfering N_2 gas can be prevented from entering the IRMS by diverting to a vent (Qi et al. 2011). Subsequently, the trapped CO is released by means of heat and then diverted to the IRMS for $\delta^{18}O$ analysis (Qi et al. 2011).

$$2AgNO_{3(s)} + 3AgCN_{(s)} \rightarrow 5Ag_{(s)} + 2.5N_{2(g)} + 3CO_{2(g)} \quad (3)$$

$$2AgNO_{3(s)} + 3C_{(s)} \rightarrow 2Ag_{(s)} + N_{2(g)} + 3CO_{2(g)} \quad (4)$$

Affecting factors and improvement of the ion-exchange method

Type of anion-exchange resins

The adsorption efficiency of nitrate depends on the type of anion-exchange resin used; different resins differ in their ion-exchange capacities. As evident in Table 3, five different types of anion-exchange resins have been investigated. Among these, the most popular resin is AG1-X8 (Cl^- form, 200–400 mesh), which exhibits excellent adsorption capacity for nitrate (Fukada et al. 2003; Silva et al. 2000). In addition, the ionic form is also an important factor affecting the adsorption efficiency. The resin in the Cl^- form has a higher adsorption efficiency than the resin in the OH^- form (Chen et al. 2010). The adsorption efficiency for anion-exchange resins in the Cl^- form vary between 97 and 99 %, whereas the adsorption efficiency is less than 90 % for the resin in the OH^- form under the same experimental conditions (Ahmed et al. 2012b; Chen et al. 2010).

Table 3 Types of anion-exchange resins used in the ion-exchange method

Description	Ionic form	Dry mesh size	Reference
AG1-X8	Cl^-	200–400	Fukada et al. 2003; Li et al. 2010; Minet et al. 2011; Silva et al. 2000; Xing and Liu 2011
Dowex 1X8	Cl^-	200–400	Ahmed et al. 2012a; Ahmed et al. 2012b
AG1X, AG2X	Cl^-	100–200	Chang et al. 1999
201×7	OH^-	50–100	Chen et al. 2010
202×7	OH^-	50–100	Chen et al. 2010
AG1-X8	Cl^-	100–200	Chen et al. 2010

Ratio of the height to the diameter of the resin bed

The height-to-diameter (H/D) ratio of the resin bed in the exchange column is also a major factor affecting the adsorption efficiency of nitrate. Researchers have two types of anion-exchange columns at their disposal: prefilled and lab-made columns. Prefilled exchange resin columns can be purchased from suppliers and are convenient; however, they are relatively expensive. To reduce costs, the resin and column can be purchased separately and the anion-exchange resin column can be constructed according to the field requirements. In addition to the type of anion-exchange resin employed, the H/D ratio of the resin bed is also a key factor for the adsorption of nitrate. As suggested from Table 4, the adsorption efficiency increases with increasing H/D ratio of the resin bed because a higher ratio leads to a longer retention time and more frequent contact between the nitrate and the resin (Ahmed et al. 2012b; Chen et al. 2010; Xing and Liu 2011). The use of the same type of exchange resin column is impractical because the nitrate concentration in freshwater varies from place to place. Therefore, if a detailed relationship between the H/D ratio in the resin bed and the nitrate concentration can be established, then a suitable exchange resin column can be selected for different samples. In this way, the efficiency and costs will be reduced accordingly. From this point of view, lab-made anion-exchange resin columns might be a better choice than those purchased from a supplier.

Flow rate of extraction

The flow rate also plays a significant role on the adsorption efficiency of nitrate, and a suitable flow rate maintains the extraction of nitrate from freshwater within a precise range. Silva et al. (2000) investigated the relationship between the flow rate and the adsorption efficiency in 87 samples with nitrate concentrations ranging from 17 to 130 mg N L^{-1}; they observed that the average nitrate adsorption efficiency was 99.84±0.16 % when the flow rate was set within a range of 90 to 565 mL h^{-1}. According to the results of statistical analysis, no correlation existed between the extent of nitrate adsorption and flow rate. Ahmed et al. (2012a, b) obtained

Table 4 Relationship between the height-to-diameter ratio and the adsorption efficiency of anion-exchange resin columns

Description	H cm	D cm	H/D	Adsorption efficiency %	Sample number *n*	Reference
Purchased	3.80	0.85	4.50	99.84±0.16	87	Fukada et al. 2003; Li et al. 2010; Minet et al. 2011; Silva et al. 2000; Xing and Liu 2011
Lab-made	2.00	1.40	1.43	95.77±1.75	3	Chen et al. 2010
Lab-made	2.50	1.40	1.78	97.83±0.85	3	Chen et al. 2010
Lab-made	3.00	1.40	2.14	98.33±0.75	3	Chen et al. 2010

similar results and observed that nearly all of the nitrate was adsorbed onto the resin when the flow rate was varied from 350 to 1500 mL h^{-1}. Irrespective of how the samples were loaded onto the resin columns—for example, by gravity, air pressing or with the aid of suction—a flow rate range of 500 to 1000 mL h^{-1} was most common. The flow rate can be adjusted by a rubber stopper in a separatory funnel or by a peristaltic pump (Chang et al. 1999; Silva et al. 2000; Xing and Liu 2011). In addition, a coarser mesh size of the same type of anion-exchange resin can be substituted to change the sample flow rate (Silva et al. 2000).

Type of eluant

Consequently, suitable eluants should maintain the elution of nitrate within a precise range. As previously mentioned, the anion-exchange resin in Cl^- form exhibited an excellent adsorption capacity for nitrate and is the most commonly used resin in this application. Therefore, chloride solutions, such as hydrochloric acid (HCl) and potassium chloride (KCl) solutions, were observed to be superior as for high nitrate yields, relative selectivity for nitrate, and its relative ease of handling (Chen et al. 2010; Silva et al. 2000). Additionally, Silva et al. (2000) and Chen et al. (2010) investigated strontium chloride ($SrCl_2$), calcium chloride ($CaCl_2$), sulfuric acid (H_2SO_4), potassium citrate ($K_3C_6H_5O_7{\cdot}2H_2O$), and potassium iodide (KI). However, these candidates were abandoned because of chemical instability or low nitrate yields.

Concentration and volume of eluants

The concentration and volume of eluants are two key factors affecting desorption efficiency, and the former is more important than the latter. As previously noted, both HCl and KCl are suitable eluants for the elution of nitrate from anion-exchange resin columns, especially HCl, which is widely used for this purpose. To achieve complete elution of nitrate from resins and save experimental costs, a proper concentration for eluant is necessary. For instance, the desorption efficiency of nitrate was maintained within a low range (lower than 85 %) along with a large variance and did not improve significantly with an increase in volume when the concentration of HCl was as low as 0.2 mol L^{-1}. The optimum concentration and volume vary from resin to resin. With respect to a 2 mL Dowex 1X8 anion-exchange resin column, 30 mL of 0.5 mol L^{-1} HCl was considered optimum for the elution of nitrate (Ahmed et al. 2012a, b). As a result, the purpose of saving cost was achieved by the reduction of acid usage. In addition, 12 to 15 mL of 3.0 mol L^{-1} HCl was most commonly used for 2 mL AG1-X8 anion-exchange resin columns (Fukada et al. 2003; Li et al. 2010; Minet et al. 2011; Silva et al. 2000; Xue et al. 2010). Furthermore, KCl is also a suitable eluant. No significant difference was noted eluted between a 40 mL of 2.0 mol L^{-1} KCl solution and a 40 mL of 3.0 mol L^{-1} HCl solution for the same sample. The KCl solution produced a slightly higher efficiency than the HCl solution (Chen et al. 2010).

Methods for eluant addition

The method of eluant addition also affects the desorption efficiency of nitrate. Silva et al. (2000) reported that the mean recovery of nitrate was greater than 95 % with a single 15 mL aliquot of 2.7 mol L^{-1} HCl but had a large variance, whereas 15 mL of 3 mol L^{-1} HCl applied in five 3 mL increments consistently recovered more than 98 % of the nitrate. Therefore, the aliquot method was adopted by researchers irrespective of type of eluant. After the eluant was added to the anion-exchange resin column, it passed through the column by gravity. To remove residual eluant, positive air pressure must be applied. As a result, a rubber bung and a peristaltic pump were introduced by Fukada et al. (2003) and Xing and Liu (2011), respectively. With the aid of the peristaltic pump, the elution speed was ten times greater than for gravity dripping when 8 mL of HCl was passed through the same anion-exchange resin column, without causing isotopic fractionation (Xing and Liu 2011).

Type of neutralization reagent

The use of Ag_2O as a neutralization reagent has several drawbacks: (1) Silver oxide is a type of costly chemical reagent (1 g Ag_2O costs approximately US$4.40); (2) commercially available Ag_2O reagent contains nitrate blank (11–75 ppm) and must be pretreated before use; (3) $AgNO_3$ is light sensitive, and its photodegradation will cause fractionation; consequently, all of the steps of elution, neutralization, and drying must be conducted quickly, and the samples must be shielded from light during preparation and during temporary storage (Ahmed et al. 2012b; Minet et al. 2011; Silva et al. 2000). Nevertheless, other alternatives (such as hydroxides, oxides, and metals) have one or more of the following fatal drawbacks: (1) Cations, in particular Na, present in the neutralization reagent react with the quartz combustion tubes during the combustion process and (2) fluffy salt (e.g., potassium chloride) generated during the neutralization process can occupy the whole combustion tube and may negatively affect the reaction products (Ahmed et al. 2012b; Silva et al. 2000). Instead of individual neutralization reagents, a combination of reagents is a better choice for achieving analytical precision. Ahmed et al. (2012b) investigated a mixed two-step neutralization scheme in which Ag_2O and KOH were chosen as neutralization reagents. They first used Ag_2O to neutralize the eluant until a pH of 1.0 was achieved and subsequently added KOH to complete the neutralization until a pH of 7.0 was reached. During the mixed neutralization procedure, majority of the original Cl^- was removed by AgCl precipitation in the first step limiting the generation of KCl decreased sharply. In this way, a cost reduction resulted from the decreased use of Ag_2O and nitrate was produced as KNO_3, which was easier to handle than the light-sensitive $AgNO_3$.

Drying methods

Freeze-drying is time-consuming and requires approximately 30 h to dry $AgNO_3$ (or KNO_3) salts (Xing and Liu 2011). To circumvent this time-consuming procedure, Fukada et al. (2003) employed an oven-drying process. They prepared ten replicates of standard solutions for processing and concluded that the oven-drying process is also a better choice for the freeze-drying method to recover $AgNO_3$, although they noted little differences between the laboratory standard process and their process. Xing et al. (2011) also investigated the effect of oven-drying on the precision of the method and observed no significant difference between the effects of oven-drying and freeze-drying on the isotope composition for nitrate tested using different types of samples (laboratory standard solutions and natural samples). Compared to freeze-drying, oven-drying is simpler and saves time (only 6 h of oven-drying at 50–60 °C is necessary to evaporate water) (Xing and Liu 2011).

Interferential anions and compounds

As evident in Table 5, the AG1-X8 anion-exchange resin exhibits a good adsorption-exchange capacity and different relative selectivity for different anions. Therefore, other anions containing in solution may play negative impact on the adsorption of nitrate by anion-exchange resin. Among these anions, chloride and sulfate are the most abundant anions and will cause isotope fractionation for nitrate analysis by competing for the adsorption sites (Silva et al. 2000). For this reason, the potential interferences from chloride, sulfate, and dissolved organic carbon (DOC) were investigated and corresponding methods to eliminate these interferences were developed.

Table 5 Relative selectivity between AG1-X8 resin and different anions (Chen et al. 2010; Silva et al. 2000)

Anion	I^-	HSO_4^-	NO_3^-	Br^-	NO_2^-	Cl^-	HCO_3^-
Relative selectivity	175	85	65	50	24	22	6

Chloride (Cl^-)

As previously mentioned, Cl^- is one of the most abundant anions in natural waters, and its relative selectivity is approximately one third that of NO_3^-. Both of NO_3^- displacement and exchange capacity exceeding resulted from Cl^- interference can lead to incomplete adsorption of NO_3^- (Silva et al. 2000). From this point of view, Cl^- is considered as potential interference. Silva et al. (2000) investigated the influence of Cl^- concentration and sample volume on nitrogen isotope fractionation with Cl^--amended NO_3^- solutions processed through the entire procedure and observed that there is a positive relationship between the extent of nitrate-nitrogen isotope fractionation and Cl^- concentration and sample volume. However, Aly et al. (2010) also conducted similar research but reported that Cl^- produced no measurable nitrate-nitrogen isotope fractionation because the significant nitrate loss resulted from the displacement of NO_3^- by Cl^- rather than exchange capacity exceeding. In addition to nitrate-nitrogen, Ahmed et al. (2012b) also investigated the effect of the chloride ion concentration on the nitrate recovery efficiency and the nitrate-oxygen fractionation; they reported that there is a negative relationship between the nitrate recovery efficiency and sample volume and chloride concentration. In addition, no significant nitrate-oxygen fractionation was found in spite of the Cl^- concentration that was as high as 4000 mg L^{-1} for 100 mL samples (Ahmed et al. 2012b).

Sulfate (SO_4^{2-}) and phosphate (PO_4^{3-})

SO_4^{2-} and PO_4^{3-} must be removed from water samples for the following reasons: (1) SO_4^{2-} is a typical competing ion because of its divalence and high selectivity and is expected to displace both NO_3^- and Cl^-; (2) both SO_4^{2-} and PO_4^{3-} are O-containing compounds and were taken as contaminants during the oxygen isotope analysis of nitrate. Silva et al. (2000) investigated the effect of SO_4^{2-} on nitrate nitrogen isotope fractionation by SO_4^{2-}-amended NO_3^- solutions processed through the entire procedure and did not observe nitrogen isotope fractionation even though the combined concentrations of NO_3^- and SO_4^{2-} exceeded the capacity of the anion-exchange resin column. However, to avoid contamination for oxygen isotope analysis, action must be taken to remove SO_4^{2-} and PO_4^{3-}. Both barium chloride ($BaCl_2$) and barium hydroxide monohydrate ($Ba(OH)_2 \cdot H_2O$) are suitable candidates for the removal of these O-bearing compounds by precipitation (Ahmed et al. 2012b; Minet et al. 2011). In the case of $BaCl_2$, the commonly used concentration is 1 mol L^{-1} and the volume varies from sample to sample. For example, 4 mL of 1 mol L^{-1} $BaCl_2$ can precipitate as much as 4 mmol of sulfate (Fukada et al. 2003; Minet et al. 2011; Xue et al. 2010). The step for removal of O-bearing contaminants by precipitation can be performed before the extraction of nitrate or after neutralization. The precipitation (such as $BaSO_4$ and $BaPO_4$) can be removed by filtration after the addition of $BaCl_2$ to the sample solution (Chang et al. 1999; Fukada et al. 2003; Li et al. 2010; Minet et al. 2011). In the case of $Ba(OH)_2 \cdot H_2O$, the saturated solution of which is added to the sample, left for 30 min, and filtered through nylon membrane filter to remove $BaSO_4$ precipitate (Ahmed et al. 2012b). In this way, the sulfate ions was removed and substituted by hydroxyl ions which lead to lower ion strength of the samples.

Dissolved organic carbon

DOC contains nitrogen and oxygen, and this content accounts for approximately 2 and 30–50 % of the average DOC molecule, respectively (Chang et al. 1999). Although the effect of DOC on the nitrogen isotope analysis of nitrate is negligible, its removal before passing the sample through the anion-exchange resin column is preferable because DOC can potentially interfere with NO_3^- adsorption onto the anion-exchange resin and may lead to the transfer of DOC oxygen into the sample (Ahmed et al. 2012b; Chang et al. 1999). Therefore, the removal of DOC is a key step for oxygen isotope analysis by the ion-exchange method (Ahmed et al. 2012b; Chang et al. 1999; Fukada et al. 2003; Silva et al. 2000; Wassenaar 1995). From the viewpoints of saving time and money, different alleviation schemes are available according to the DOC and nitrate concentrations in the water sample. For samples containing low to moderate DOC concentrations (0.08–0.8 mmol L^{-1}), a special alleviation scheme to remove DOC is unnecessary because most of the DOC is attenuated by adsorption, precipitation, or volatilization throughout the experimental procedure (Silva et al. 2000). For samples containing a high DOC concentration, DOC removal can be achieved with cation-exchange resins, activated-charcoal adsorption columns, or activated carbon (Ahmed et al. 2012b; Chang et al. 1999; Silva et al. 2000). The use of a cation column prior to an anion column reduces the possibility of anion-exchange resin to retain DOC. However, the removal efficiency varied from resin to resin and would likely vary with the local DOC composition (Ahmed et al. 2012b). In order to remove more DOC, activated charcoal column (coarse grained) and activated carbon can be employed. Compared to an activated charcoal column (coarse grained), activated carbon is effective, convenient to handle, and cheap (Ahmed et al. 2012b). However, activated carbon can adsorb nitrate if applied in an excessively high concentration, and its adsorption efficiency is affected by numerous factors, including the additional amount of activated carbon, the contact time with the water sample, the shaking speed, etc. Therefore, to remove DOC without sacrificing nitrate, proper experimental conditions must be used. The optimum conditions for DOC removal while leaving the nitrate intact were 20 mg Norit G-60 activated carbon per 100 mL water sample and then shaking 20 min (Silva et al. 2000). However, activated carbon and activated charcoal would cause O-18 discrimination for nitrate pool in the mobile phase; this fractionation amounted to −6 and +10‰, respectively (Ahmed et al. 2012a, b).

Sample preservation

Before samples are loaded onto an anion-exchange resin column, the samples must be carefully preserved to preserve the nitrate pools. Ahmed et al. (2012b) investigated the effect of single or combined use of chloroform, chilling, and freezing on the nitrate pools of artificial wastewater and observed no significant change in the $\delta^{18}O$ values for nitrate in samples treated with chloroform (addition of 0.05 to 1000 mL sample), irrespective of the temperature conditions (chilling, freezing, or room temperature). After the sample was loaded onto an anion-exchange resin column and stored in a moist and refrigerated environment, no significant difference was observed for $\delta^{15}N$ values of nitrate between stored and directly processed samples after preservation for 7 months to 2 years (Ahmed et al. 2012b; Silva et al. 2000). After being eluted from the anion-exchange resin column by HCl, the eluant must be quickly neutralized. Otherwise, slight isotope fractionation might occur due to HNO_3 evaporation (Silva et al. 2000). Therefore, to avoid isotope fractionation and reduce the use of toxic chemical compounds (e.g., $CHCl_3$ or $HgCl_2$), samples must be processed as soon as possible (Silva et al. 2000).

Best procedure available now for ion-exchange method

Sampling and storage

Water samples are collected from freshwater ecosystems (e.g., streamwater, river) with a depth-integrating sampler according to the United States Geological Survey (USGS) protocols (Battaglin et al. 2001; Chang et al. 2002). To satisfy the requirements for dual isotope analysis (approximately 1.4–2.8 mg N), it is firstly needed to determine NO_3^- concentrations and then confirm the quantity of water sample for processing (Ahmed et al. 2012b; Silva et al. 2000). In addition, the concentrations of Cl^-, SO_4^{2-}, PO_4^{3-}, HCO_3^-, and DOC also need to be determined. Subsequently, the water samples are filtered through a 0.45 μm membrane into precleaned polyethylene bottles to remove particles. Addition of chloroform (99.8 %) with the ratio of 0.25 mL L^{-1} water sample prevents nitrate isotopic fractionation caused by microbial activities (Ahmed et al. 2012b). The water samples can subsequently be shipped to the laboratory for further processing.

Pretreatment

Sulfate (SO_4^{2-}), phosphate (PO_4^{3-}), and dissolved organic carbon (DOC) are the most abundant materials that will cause isotope fractionation for nitrate analysis by competing for the adsorption sites (Silva et al. 2000). Therefore, water samples must be pretreated before the next step. Addition of saturated $Ba(OH)_2 \cdot H_2O$ to water sample, precipitated for half an hour, and filtered by 0.2 mm nylon filter to remove precipitated $BaSO_4$ and $Ba_3(PO_4)_2$ (Ahmed et al. 2012b; Fukada et al. 2003). Then, the cation-exchange column packed with resin (such as Bio-Rad AG 50W-X8) can be employed to remove DOC and residual Ba^{2+} (Panno et al. 2008).

Extraction

In this step, the pretreated water sample was passed through the anion-exchange columns at a flow rate of 500 to 1000 mL h^{-1} (Fukada et al. 2003). In this way, nitrate is extracted from water and retained onto resins. Because incomplete adsorption leads to nitrate fractionation, the NO_3^- adsorption efficiency must be verified by determination of the NO_3^- concentration in the eluant passed through the anion-exchange columns. Extraction requires at least 95 % efficiency to avoid significant fractionation (Battaglin et al. 2001; Silva et al. 2000).

Elution

After the extraction step, the NO_3^- is stripped from the anion-exchange resin by gravity dripping of 15 mL of 3 mol L^{-1} HCl in five 3 mL increments and then collected the 15 mL of nitrate-bearing eluant by 50 mL glass beakers at the same time (Silva et al. 2000). To remove residual eluant, a rubber bung can be employed to supply positive air pressure to the column after each HCl addition (Fukada et al. 2003). With this approach, more than 98 % of the nitrate can be recovered from the anion-exchange resin (Silva et al. 2000). With respect to a 2 mL Dowex 1X8 anion-exchange resin column, 30 mL of 0.5 mol L^{-1} HCl was considered optimum for the elution of nitrate (Ahmed et al. 2012a, b).

Neutralization

Nitric acid (HNO_3) and HCl coexist in the nitrate-bearing eluant from the anion-exchange resin column (Eq. 1) (Ahmed et al. 2012a, b). Because HNO_3 is volatile and isotopic fractionation will occur under low-pH conditions, it must be neutralized as soon as possible before the drying process (Ahmed et al. 2012b; Böhlke et al. 2003; Silva et al. 2000). To prevent the loss of nitrate, the eluant is neutralized immediately with silver oxide (Ag_2O), which must be rinsed to remove any traces of NO_3^- before use. The beaker containing 15 mL of nitrate-bearing eluant is placed in cold-water bath, and Ag_2O (prewashed to remove contaminant NO_3^-) is added into the beaker increments to neutralize the HNO_3 and HCl until a pH of 1 is achieved in the final solution and subsequently added KOH to complete the neutralization until a pH of 7.0 was reached. At last, the solution was filtered to remove silver chloride precipitate (Ahmed et al. 2012a, b; Silva et al. 2000).

Drying and stable isotope analysis

Nitrogen isotopic analysis

To obtain solid KNO_3 salts, neutralized solution consisting of KNO_3 and KCl was evaporated till complete dryness on a hot plate. The dried salt mix was mixed with activated graphite (in 1:1 mass ratio to KNO_3) and finally ground. Then, the mixture is put into a 20 cm length and 6 mm (OD) Pyrex glass tubes which need to be precombusted, and the combustion tubes are evacuated, torch-sealed, combusted, and cooled (Ahmed et al. 2012b). In this way, the KNO_3 samples were completely decomposed and the liberated CO_2 gas was available for oxygen isotopic analysis by IRMS (Ahmed et al. 2012a, b; Aly et al. 2010). The nitrogen isotopic ratios (R) are reported as the parts per thousand (or per mil, ‰) deviation from the $^{15}N/^{14}N$ ratios relative to N_2 (air). To verify the analytical accuracy, internationally accepted reference materials supplied by USGS and the International Atomic Energy Agency (IAEA) can be employed for isotope analysis (e.g., USGS25, IAEA-N-1, and IAEA-N-2) (Minet et al. 2011; Zhang et al. 2014).

Oxygen isotopic analysis

Activated carbon should be employed to remove residual DOC, and the optimum conditions for DOC removal while leaving the nitrate intact were 20 mg Norit G-60 activated carbon per 100 mL water sample and then shaking 20 min, filtered by 0.2-μm nylon filter to remove residual SO_4^{2-}. Then, the cation-exchange column packed with resin (such as Bio-Rad AG 50W-X8) can be employed to remove the residual Ba^{2+} (Panno et al. 2008). After re-neutralized, the solution was dried by hot plate. The dried salt mix was mixed with activated graphite (in 1:1 mass ratio to KNO_3) and finally ground. Then, the mixture is put into a 20 cm length and 6 mm (OD) Pyrex glass tubes which need to be precombusted, and the combustion tubes are evacuated, torch-sealed, combusted, and cooled (Ahmed et al. 2012b). In this way, the KNO_3 samples were completely decomposed and the liberated CO_2 gas was available for oxygen isotopic analysis by IRMS. The oxygen isotopic ratios (R) are reported as the parts per thousand (or per mil, ‰) deviation from the $^{18}O/^{16}O$ ratios relative to standard mean ocean water (SMOW). To verify the analytical accuracy, internationally accepted reference materials supplied by USGS and the International Atomic Energy Agency (IAEA) can be employed for isotope analysis (e.g., USGS32, USGS34, and IAEA-NO-3) (Minet et al. 2011; Zhang et al. 2014).

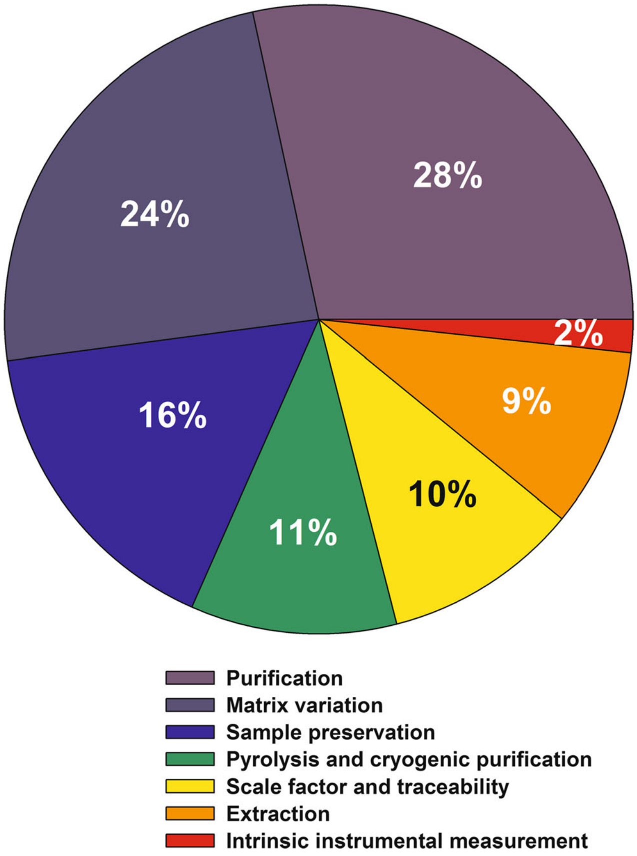

Fig. 2 Uncertainty contributors to the total uncertainty budget of the ion-exchange method (adapted from Ahmed et al. 2012b)

Discussion and perspective

To date, the ion-exchange method has been widely employed in the collection of nitrate from freshwater ecosystems for nitrogen and oxygen isotope analysis. However, this method has inherent shortcomings, such as high costs and a negative influence of interfering ions (such as chloride, sulfate, etc.); thus, appropriate modifications must be made to this method. First of all, the uncertainties associated with the method and which step gives large contribution should be understood and paid more attention. According to the uncertainty evaluation performed by Ahmed et al. (2012b), the step of purification and DOC removal contributes the most to the total uncertainty budget, followed by the step of matrix variation effects and sample preservation (Fig. 2). Therefore, in order to improve the overall accuracy of the method, the step of purification and DOC removal need to be paid more attention in the future. In addition, the validation and performance of the modified ion-exchange method should be verified by well-established method by determination of large sample sizes at several laboratories (Ahmed et al. 2012b). Time-consuming is also a drawback of the ion-exchange method. Compared to the procedure for measuring $\delta^{15}N$, it is more time-consuming for measuring $\delta^{18}O$ since there is a great need to eliminate the negative impact of oxygen-bearing anions and DOC. According to the method characteristics evaluated by Aly et al. (2010), one sample for $\delta^{15}N$ analysis of nitrate requires 1–2 h for complete processing from field to IRMS in a set of five samples. However, it will cost at least 4–6 days for $\delta^{18}O$ analysis of nitrate for five samples in triplicate means (Ahmed et al. 2012b). Therefore, removal of interfering ions, such as SO_4^{2-} and DOC, is needed to be improved in the future research. Another drawback to the ion-exchange method is its high costs. Therefore, cost reduction is a key step to encourage the application of the ion-exchange method. How can costs be reduced through modification of the ion-exchange method? The total consumables alone for the ion-exchange method developed by Silva et al. (2000) can cost as much as US$76.9 per sample for nitrogen and oxygen stable isotope analysis in duplicate measurements (Xue et al. 2009). While a reduction of 40 % of the total consumables cost was achieved for a modified ion-exchange method developed by Ahmed et al. (2012b). The cost of the column resins (both cation and anion) and the neutralization reagent (Ag_2O) account for more than 60 % of the total cost of consumables (Fig. 3). For the modified method by Ahmed et al. (2012b), the anion and cation exchange column was made by injection syringe and resins rather than commercial columns (about US$9.4 per commercial anion or cation exchange resins column). Taking these factors into account, nearly 90 % of the cost would be paid on the column resins (both cation and anion) and Ag_2O. Thus, cost savings can be achieved through modification of the use of these consumables. Self-made resin columns are a good choice to replace commercial prefilled columns. Columns and resin of the same pattern as commercial columns can be purchased separately and used to make columns similar to the prefilled columns (Silva et al. 2000). In addition, a sterile syringe is also an alternative for commercial empty

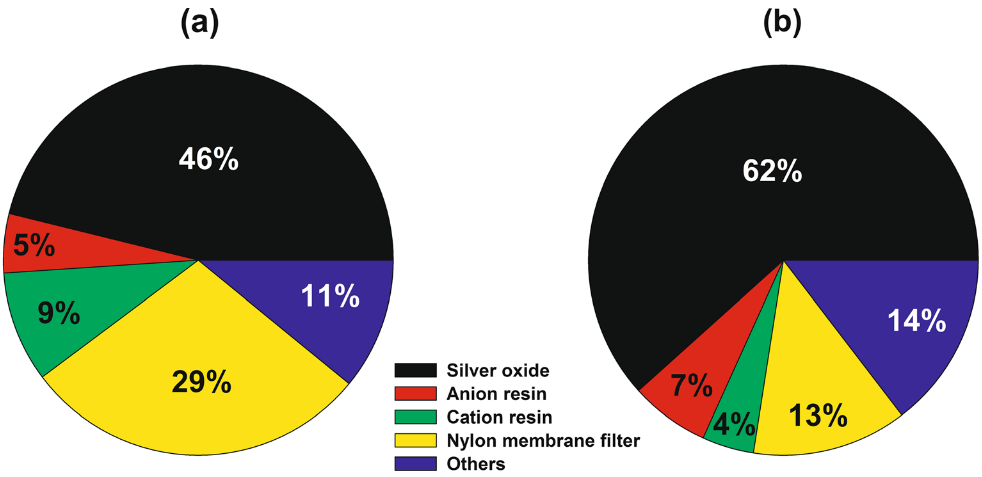

Fig. 3 Cost analysis of consumables for the ion-exchange method (adapted from Ahmed et al. 2012b)

column (Ahmed et al. 2012b; Xing and Liu 2011). These two approaches can save as high as US$26 per sample. Nonetheless, a detailed relationship between the H/D ratio in the resin bed and the nitrate concentration needs to be established; then, a suitable exchange resin column can be selected for different samples. In this way, the efficiency and costs will be reduced accordingly. Regarding another costly consumable, Ag_2O, cost savings can be realized in three ways: (1) reducing the amount of Ag_2O. Xing and Liu (2011) compared a 1 mL sterile syringe exchange resin column with a commercial prefilled column and observed that 2.5–3.0 g Ag_2O could be saved, reducing the cost by US$4.30 per sample; (2) the development of an alternative neutralization reagent. Compared to the use of Ag_2O alone, combined neutralization with Ag_2O and KOH can also lower the costs (Ahmed et al. 2012b); (3) regeneration of Ag_2O. The AgCl waste from the neutralization process can be converted back to Ag_2O through a series of chemical reactions (Silva et al. 2000). With this approach, Ag_2O is no longer a consumable. Although Silva et al. (2000) did not compare the costs between regenerating and purchasing Ag_2O, regenerating Ag_2O might be a better choice because Ag is a precious metal, which should be reused instead of disposed of as waste. If the cost can be significantly reduced, more information can be obtained for the same cost. For instance, the ion-exchange method can be employed for long-term monitoring of dual stable changes in freshwater systems and can reveal the characteristics of dynamic changes and the potential driving force in the freshwater system. In addition, will the interaction of interfering ions (such as chloride or sulfate) affect the extraction efficiency of nitrate? Adsorption of nitrate onto anion-exchange resins can be interfered by high concentrations of anions in water samples. The effect of interfering anions (such as chloride or sulfate) on the adsorption of nitrate onto anion-exchange resins has been investigated because they could lead to nitrate isotope fractionation. In these experiments, the influence of each interfering ion on nitrate recovery and isotope fractionation was individually investigated, and the authors concluded that no significant isotope fractionation occurred when the interfering anion concentration was within a particular range. However, these results do not agree with reality because interfering anions coexist in water samples. Therefore, the comprehensive influence of interfering ions on nitrate recovery and isotope fractionation should be investigated in the future; in addition, the extent and order of different types of interfering anions on the analytical results can be fully understood, and corresponding methods to offset their effects can be implemented accordingly. In this case, the ion-exchange method can not only be used for the investigation of clean water samples (e.g., surface water and groundwater) but can also be employed to investigate water samples containing complex constituents (e.g., landfill leachate). Landfill leachate is a complex mixture that contains ammonium nitrogen, nitrate, and heavy metals (Kjeldsen et al. 2002). Because heavy metals affect bacterial cultivation, the bacterial denitrification method is not suitable for stable isotope analysis of nitrate in landfill leachate. The passive diffusion method was used as an alternative method for the determination of $\delta^{15}N–NO_3^-$ in landfill leachates (North et al. 2004). However, the passive diffusion method has some drawbacks, such as being time-consuming, and has uncertainties regarding isotope fractionation and lower analytical accuracy. Therefore, if countermeasures to relieve the interference of high concentrations of anions with the adsorption of nitrate onto anion-exchange resins are available, more information on the fate of nitrate in landfill leachate can be revealed. Finally, in order to verify whether the samples can be archived on the resin columns, Ahmed et al. (2012b) and Silva et al. (2000) conducted nitrogen and oxygen isotope fractionation experiments, respectively. According to their results, no significant isotope fractionation occurred when nitrate was preserved on the anion-exchange resin columns for 7 months (for oxygen) and up to 2 years (for nitrogen). Although nitrogen and oxygen isotope fractionation was not investigated in the same experiment, no significant isotope fractionation was expected after the samples were archived on the resin columns for at least 7 months. Therefore, the ion-exchange method can be used as an alternative sample preservation technique for other analytical techniques for the determination of $\delta^{15}N–NO_3^-$ and $\delta^{18}O–NO_3^-$, such as the bacterial denitrification method. Nitrate in freshwater samples can be placed onto anion-exchange resins in the field and shipped to the laboratory, where the nitrate can be stripped for further processing. However, the use of Ag_2O as neutralizing agent is impractical since the silver ions have antimicrobial activity (Chopra 2007). Under this circumstance, KOH is a suitable alternative for Ag_2O to neutralize the eluant till a pH of 7.0 was achieved. In this way, the nitrate is extracted from the resin columns and converted into KNO_3 salts, which are subsequently processed for stable isotope analysis by the bacterial denitrification method. As a result, two advantages can be realized: (1) enrichment of nitrate for low-nitrate-containing freshwater samples and (2) elimination of the bias of isotopic composition of N_2O produced from NO_2^- in water samples by the bacterial denitrification method because the relative selectivity of NO_2^- is approximately one third that of NO_3^- (Table 5).

Conclusions

The ion-exchange method is a reliable and precise technique for measuring $\delta^{15}N$ and $\delta^{18}O$ in NO_3^- and plays an important role in distinguishing the sources of nitrate contamination for freshwater ecosystems. This review summarizes and presents the principles, affecting factors and corresponding significant improvements of the ion-exchange method. The modification of the method or the development of alternative consumables such as columns, resins, and neutralization reagents is necessary to reduce costs and increase the applicability of this method. In addition, to enlarge the applicable range of this method, the effect of interfering ions on the efficiency of this method and corresponding methods to offset these effects should be investigated and the function of sample preservation for stable analysis needs to be confirmed in the future. In comparison to other analytical techniques for the determination of $\delta^{15}N–NO_3^-$ and $\delta^{18}O–NO_3^-$, more accurate analysis results can be attained using the anion column technique. These research results will be particularly valuable in revealing dynamic change characteristics and the potential driving forces in freshwater systems and in providing information on the fate of nitrate in other liquid samples, such as landfill leachates.

Acknowledgments This study was made possible through a grant from the National Natural Science Foundation of China (41401556, 31261120580, and 31400346), Key Technology Research and Development Program of Science and Technology Department in Zhejiang province (2012C13012) and Scientific Research Foundation for Young Scholars, Hangzhou Normal University (PD12002004131).

Conflict of Interest The authors declare that they have no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

References

Ahmed M, Aly A, Gomaa H (2012a) Developed method for nitrate extraction and purification to measure $\delta^{18}O-NO_3^-$ composition in water. Arab J Nucl Sci Appl 45:1–13

Ahmed M, Aly A, Abdel Monem N, Hanafy M, Gomaa H (2012b) A modified procedure for measuring oxygen-18 content of nitrate. J Hydrol 472:193–204

Aly AIM, Ahmed MA, Gomaa HE, Abdel Monem N, Hanafy M (2010) Rapid and accurate technique for measuring N-15 of nitrate method development, calibration and validation. Isotope Rad Res 42:39–56

Aravena R, Robertson WD (1998) Use of multiple isotope tracers to evaluate denitrification in ground water: study of nitrate from a large-flux septic system plume. Groundwater 36:975–982

Aravena R, Evans M, Cherry JA (1993) Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. Groundwater 31:180–186

Bateman AS, Kelly SD (2007) Fertilizer nitrogen isotope signatures. Isot Environ Healt S 43:237–247

Battaglin WA, Kendall C, Chang CC, Silva SR, Campbell D (2001) Chemical and isotopic evidence of nitrogen transformation in the Mississippi River, 1997–98. Hydrol Process 15:1285–1300

Böhlke J, Mroczkowski S, Coplen T (2003) Oxygen isotopes in nitrate: new reference materials for $^{18}O:^{17}O:^{16}O$ measurements and observations on nitrate-water equilibration. Rapid Commun Mass Sp 17: 1835–1846

Boumans L, Fraters D, van Drecht G (2004) Nitrate leaching by atmospheric N deposition to upper groundwater in the sandy regions of the Netherlands in 1990. Environ Monit Assess 93:1–15

Burgin AJ, Hamilton SK (2007) Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Front Ecol Environ 5:89–96

Camargo JA, Alonso Á (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ Int 32:831–849

Camargo JA, Alonso A, Salamanca A (2005) Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere 58:1255–1267

Casciotti K, Sigman D, Hastings MG, Böhlke J, Hilkert A (2002) Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. Anal Chem 74: 4905–4912

Chang CC, Langston J, Riggs M, Campbell DH, Silva SR, Kendall C (1999) A method for nitrate collection for $\delta^{15}N$ and $\delta^{18}O$ analysis from waters with low nitrate concentrations. Can J Fish Aquat Sci 56:1856–1864

Chang CC, Kendall C, Silva SR, Battaglin WA, Campbell DH (2002) Nitrate stable isotopes: tools for determining nitrate sources among different land uses in the Mississippi River Basin. Can J Fish Aquat Sci 59:1874–1885

Chen W, Chen W, Rao H, Zhang L, Hong H (2010) An improved ion-exchange/diffusion method for $\delta^{15}N$ isotope tracing analysis of nitrate in surface waters from watersheds. J Environ Sci 22:784–788

Chen ZX, Yu L, Liu WG, Lam MH, Liu GJ, Yin XB (2014) Nitrogen and oxygen isotopic compositions of water-soluble nitrate in Taihu Lake water system, China: implication for nitrate sources and biogeochemical process. Environ Earth Sci 71:217–223

Choi WJ, Ro HM, Lee SM (2003) Natural ^{15}N abundances of inorganic nitrogen in soil treated with fertilizer and compost under changing soil moisture regimes. Soil Biol Biochem 35:1289–1298

Choi WJ, Han GH, Lee SM, Lee GT, Yoon KS, Choi SM, Ro HM (2007) Impact of land-use types on nitrate concentration and δ^{15}N in unconfined groundwater in rural areas of Korea. Agr Ecosyst Environ 120:259–268

Chopra I (2007) The increasing use of silver-based products as antimicrobial agents: a useful development or a cause for concern? J Antimicrob Chemoth 59:587–590

Deutsch B, Kahle P, Voss M (2006) Assessing the source of nitrate pollution in water using stable N and O isotopes. Agron Sustain Dev 26:263–267

Di H, Cameron K (2002) Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. Nutr Cycl Agroecosys 64: 237–256

Ding J, Xi B, Gao R, He L, Liu H, Dai X, Yu Y (2014) Identifying diffused nitrate sources in a stream in an agricultural field using a dual isotopic approach. Sci Total Environ 484:10–18

Fukada T, Hiscock KM, Dennis PF, Grischek T (2003) A dual isotope approach to identify denitrification in groundwater at a river-bank infiltration site. Water Res 37:3070–3078

Huber B, Bernasconi SM, Pannatier EG, Luster J (2012) A simple method for the removal of dissolved organic matter and δ^{15}N analysis of NO_3^- from freshwater. Rapid Commun Mass Sp 26:1475–1480

Kjeldsen P, Barlaz MA, Rooker AP, Baun A, Ledin A, Christensen TH (2002) Present and long-term composition of MSW landfill leachate: a review. Crit Rev Environ Sci Tec 32:297–336

Kohl DH, Shearer GB, Commoner B (1971) Fertilizer nitrogen: contribution to nitrate in surface water in a corn belt watershed. Science 174:1331–1334

Kornexl BE, Gehre M, Hofling R, Werner RA (1999) On-line δ^{18}O measurement of organic and inorganic substances. Rapid Commun Mass Sp 13:1685–1693

Lee KS, Bong YS, Lee D, Kim Y, Kim K (2008) Tracing the sources of nitrate in the Han River watershed in Korea, using δ^{15}N–NO_3^- and δ^{18}O–NO_3^- values. Sci Total Environ 395:117–124

Lenihan HS, Peterson CH (1998) How habitat degradation through fishery disturbance enhances impacts of hypoxia on oyster reefs. Ecol Appl 8:128–140

Li X, Masuda H, Koba K, Zeng H (2007) Nitrogen isotope study on nitrate-contaminated groundwater in the Sichuan Basin, China. Water Air Soil Poll 178:145–156

Li SL, Liu CQ, Li J, Liu X, Chetelat B, Wang B, Wang F (2010) Assessment of the sources of nitrate in the Changjiang River, China using a nitrogen and oxygen isotopic approach. Environ Sci Technol 44:1573–1578

Liang X, Nie Z, He M, Guo R, Zhu C, Chen Y, Stephan K (2013) Application of ^{15}N–^{18}O double stable isotope tracer technique in an agricultural nonpoint polluted river of the Yangtze Delta Region. Environ Sci Pollut R 20:6972–6979

Liu CQ, Li SL, Lang YC, Xiao HY (2006) Using δ^{15}N- and δ^{18}O- values to identify nitrate sources in karst ground water, Guiyang, Southwest China. Environ Sci Technol 40:6928–6933

Liu T, Wang F, Michalski G, Xia X, Liu S (2013) Using ^{15}N, ^{17}O, and ^{18}O to determine nitrate sources in the Yellow River, China. Environ Sci Technol 47:13412–13421

Liu MZ, Seyf-Laye A-SM, Ibrahim T, Gbandi D-B, Chen HH (2014) Tracking sources of groundwater nitrate contamination using nitrogen and oxygen stable isotopes at Beijing area, China. Environ Earth Sci 72:707–715

Mallin MA, Johnson VL, Ensign SH, MacPherson TA (2006) Factors contributing to hypoxia in rivers, lakes, and streams. Limnol Oceanogr 51:690–701

Mayer B, Bollwerk SM, Mansfeldt T, Hütter B, Veizer J (2001) The oxygen isotope composition of nitrate generated by nitrification in acid forest floors. Geochim Cosmochim Ac 65:2743–2756

Mayer B, Boyer EW, Goodale C, Jaworski NA, Van Breemen N, Howarth RW, Seitzinger S, Billen G, Lajtha K, Nadelhoffer K (2002) Sources of nitrate in rivers draining sixteen watersheds in the northeastern US: isotopic constraints. Biogeochemistry 57: 171–197

McIlvin MR, Altabet MA (2005) Chemical conversion of nitrate and nitrite to nitrous oxide for nitrogen and oxygen isotopic analysis in freshwater and seawater. Anal Chem 77:5589–5595

Michalski G, Meixner T, Fenn M, Hernandez L, Sirulnik A, Allen E, Thiemens M (2004) Tracing atmospheric nitrate deposition in a complex semiarid ecosystem using Δ^{17}O. Environ Sci Technol 38: 2175–2181

Minet E, Goodhue R, Coxon CE, Kalin RM, Meier-Augenstein W (2011) Simplifying and improving the extraction of nitrate from freshwater for stable isotope analyses. J Environ Monitor 13:2062–2066

North JC, Frew RD, Peake BM (2004) The use of carbon and nitrogen isotope ratios to identify landfill leachate contamination: Green Island Landfill, Dunedin, New Zealand. Environ Int 30:631–637

Panno SV, Hackley KC, Kelly WR, Hwang H-H (2006) Isotopic evidence of nitrate sources and denitrification in the Mississippi River, Illinois. J Environ Qual 35:495–504

Panno SV, Kelly WR, Hackley KC, Hwang H-H, Martinsek AT (2008) Sources and fate of nitrate in the Illinois River Basin, Illinois. J Hydrol 359:174–188

Pardo LH, Kendall C, Pett-Ridge J, Chang CC (2004) Evaluating the source of streamwater nitrate using δ^{15}N and δ^{18}O in nitrate in two watersheds in New Hampshire, USA. Hydrol Process 18: 2699–2712

Qi H, Coplen TB, Wassenaar LI (2011) Improved online δ^{18}O measurements of nitrogen- -and sulfur-bearing organic materials and a proposed analytical protocol. Rapid Commun Mass Sp 25:2049–2058

Rabalais NN (2002) Nitrogen in aquatic ecosystems. Ambio 31:102–112

Rock L, Ellert BH (2007) Nitrogen-15 and oxygen-18 natural abundance of potassium chloride extractable soil nitrate using the denitrifier method. Soil Sci Soc Am J 71:355–361

Russell KM, Galloway JN, Macko SA, Moody JL, Scudlark JR (1998) Sources of nitrogen in wet deposition to the Chesapeake Bay region. Atmos Environ 32:2453–2465

Sigman D, Casciotti K, Andreani M, Barford C, Galanter M, Böhlke J (2001) A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Anal Chem 73:4145–4153

Silva S, Kendall C, Wilkison D, Ziegler A, Chang C, Avanzino R (2000) A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen isotope ratios. J Hydrol 228:22–36

Smith VH (2003) Eutrophication of freshwater and coastal marine ecosystems a global problem. Region. Environ Sci Pollut R 10:126–139

Thorburn PJ, Biggs JS, Weier KL, Keating BA (2003) Nitrate in groundwaters of intensive agricultural areas in coastal Northeastern Australia. Agr Ecosyst Environ 94:49–58

Wassenaar LI (1995) Evaluation of the origin and fate of nitrate in the Abbotsford Aquifer using the isotopes of ^{15}N and ^{18}O. Appl Geochem 10:391–405

Widory D, Petelet-Giraud E, Négrel P, Ladouche B (2005) Tracking the sources of nitrate in groundwater using coupled nitrogen and boron isotopes: a synthesis. Environ Sci Technol 39:539–548

Williard KW, DeWalle DR, Edwards PJ, Sharpe WE (2001) ^{18}O isotopic separation of stream nitrate sources in mid-Appalachian forested watersheds. J Hydrol 252:174–188

Xing M, Liu W (2011) An improved method of ion exchange for nitrogen isotope analysis of water nitrate. Anal Chim Acta 686:107–114

Xue D, Botte J, De Baets B, Accoe F, Nestler A, Taylor P, Van Cleemput O, Berglund M, Boeckx P (2009) Present limitations and future prospects of stable isotope methods for nitrate source identification in surface-and groundwater. Water Res 43:1159–1170

Xue D, De Baets B, Botte J, Vermeulen J, Van Cleemput O, Boeckx P (2010) Comparison of the silver nitrate and bacterial denitrification methods for the determination of nitrogen and oxygen isotope ratios of nitrate in surface water. Rapid Commun Mass Sp 24:833–840

Yuan L, Pang Z, Huang T (2012) Integrated assessment on groundwater nitrate by unsaturated zone probing and aquifer sampling with environmental tracers. Environ Pollut 171:226–233

Zhang Y, Liu X, Fangmeier A, Goulding K, Zhang F (2008) Nitrogen inputs and isotopes in precipitation in the North China Plain. Atmos Environ 42:1436–1448

Zhang Y, Li F, Zhang Q, Li J, Liu Q (2014) Tracing nitrate pollution sources and transformation in surface- and ground-waters using environmental isotopes. Sci Total Environ 490:213–222