An improved ion-exchange/diffusion method for ^{15}N isotope tracing analysis of nitrate in surface waters from watersheds

Weiqi Chen[1,2,3,*], Weicai Chen[2], Huanhuan Rao[2],
Luoping Zhang[1,2,3], Huasheng Hong[1,2,3]

1. State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China.
E-mail: wqchen@xmu.edu.cn
2. Environmental Science Research Center, Xiamen University, Xiamen 361005, China.
3. Coastal and Ocean Management Institute, Xiamen University, Xiamen 361005, China

Abstract

An improved method, suitable for collecting nitrate from surface waters in the watershed for ^{15}N isotope tracing analysis, was developed on the basis of the anion exchange coupled with diffusion through systematic simulation and comparison experiments. The results showed that the nitrate could be separated and enriched from the waters efficiently by using the improved method. Being simple and practical in operation principle and procedures, cost-economic, and highly efficient in nitrate separation/enrichment, the method met the requirements of δ^{15}N mass spectrum analysis and would lay a foundation for the application of ^{15}N isotope tracing approach to the research on non-point source pollution in watershed.

Key words: anion exchange and diffusion; nitrate; ^{15}N tracing approach; surface water; watershed

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Introduction

The problems of non-point pollution, on the part of agriculture particularly, have been gradually highlighted as the increasing level of industrial point pollution control (Bao and Wang, 1996; Xu, 1996). Non-point pollution is resulted from non-point discharge, including urban and agricultural runoff arising from rainfall, irrigation, precipitation and ice-melting, etc. Surface runoff is the main medium of transport for dissolved or solid non-point source pollutants. Agricultural non-point pollution is mainly caused by agricultural activities and the pollutants, including organic or inorganic substances covering the sources of soil granule, nitrogen and phosphorus, compost, pesticide and manure from livestock, are introduced into a surface water body or an aquifer by surface runoff, field drainage, underground leakage and soil erosion, etc. Being affected by multiple factors such as human activity, geography, climate, characteristics of broad origins, high randomicity, complex originality, and the uncertainty of the types and quantity, such pollutants are difficult to be identified or distinguished (Curt et al., 2004).

^{15}N tracing technique offers an approach to track the source of nitrate, an important non-point agricultural pollutant of surface runoff in watershed (Mayer et al., 2002; Spoelstra et al., 2001; Chang et al. 2002; Dai and Sun,

2007). However, the applications of this technique have been limited because of the lack of matured sample-processing method. Commonly, in order to measure ^{15}N isotope of nitrate in surface runoff from a watershed, it requires mostly filtration of water sample, separation, enrichment, transformation and purification of nitrate (Lqbal et al., 1997). As newly-developed mass spectrometer combines the device of transformation and purification with mass-spectrometer sampling system, isotope analysis can be done by directly introducing the nitrate into the system following its separation/enrichment from the water sample. For this reason, the separation and enrichment of nitrate from water sample has become the most important step for accurate analysis of ^{15}N isotope by mass spectrometry. This is particularly true for surface runoff waters as the nitrate concentrations in such waters are generally low (Karr et al., 2003). Current methods, developed for nitrate separation/enrichment based on ion exchange, distillation, and/or diffusion etc. (Xiao and Liu, 2001; Zhou et al., 2006), have some flaws to different extent and are not satisfactorily applicable, in particular to the surface runoff of watersheds.

This study aims at ameliorating the method of ion exchange by combining it with diffusion method, and tries to establish a protocol of sample preparation which is applicable to the analysis of ^{15}N in nitrate of the surface runoff from watershed. It is anticipated that this study

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* Corresponding author. E-mail: wqchen@xmu.edu.cn

will lay a foundation for expanding the application of ^{15}N isotope tracing technique for study of non-point pollution in runoff.

1 Materials and methods

1.1 Equipments, reagents, and methods for anion exchange experiments

Standard method was applied to determine nitrate concentration of water sample at the wavelength of 220 nm to 275 nm using UV-Visible spectrophotometer (HP8453, HP, USA) .

Water sample was vacuum-filtered through a 0.45-μm cellulose acetate membrane to remove insoluble particles using vacuum-filtration devices (500 mL).

Anion exchange resins, 201 × 7 (OH-form, 50–100 mesh, Shanghai Resin Factory, China), 202 × 7 (OH-form, 50–100 mesh, Shanghai Resin Factory, China) and Bio-Rad AG1-X8 (Cl-form, 100–200 mesh, Bio-Rad Laboratories, Inc., USA), were used. The resins were preconditioned through washing 3 times with Milli-Q water, soaking with 1 mol/L NaOH for 1.5–2 hr, followed by washing to neutral pH with Milli-Q water and soaking with 3 mol/L HCl for another 1.5–2 hr.

A lab-made upper-wide-bottom-narrow glass column with 10 cm length × 2.5 cm inner diameter on the top and 5 cm length × 1.4 cm inner diameter in the bottom was used for preparing exchange column. The mixture of preconditioned resin and hydrochloric acid was loaded into the glass column after a pledget was placed in the bottom to prevent leakage of resin, and the ion-exchange resin was made to reach a desired height. At the end, Milli-Q water was added into the column to wash the resin to neutral pH, and the water level was higher than the resin bed to prevent the resin from drying.

Reagents used in this study (HCl, KCl, KI, and KNO$_3$) were all GR grade and prepared to required concentrations with Milli-Q water.

Al$_2$O$_3$ column was prepared as follows. Alumina powder (100–200 mesh, neutral, chromatographic, Sinopharm Chemical Reagent Co., Ltd., China) was washed 3 times with Milli-Q water and loaded into a glass column (absorbent cotton was placed in the bottom of the column to prevent the alumina powder from leaking) with Milli-Q water, to make an alumina chromatographic column with 2.5 cm height. The column was used for the experiments of the improved ion-exchange method.

1.2 Equipments, reagents, and methods for diffusion experiments

Magnesia powder (AR grade) was used to keep solution alkaline. Concentrated sulfuric acid was used as absorbent. Chemical reagent-Devardas Alloy (AR grade, Beijing Hengye Zhongyuan Chemical Co., Ltd., China) was triturated into 100–200 mesh in agate mortar and then sealed as reducing agent.

Thermostat bath oscillator (ZD-85, Shanghai Jinpeng Analytical Instrument Co., Ltd., China) was used.

Polypropylene plastic bottles (250 mL) with inner cork were cleaned with Milli-Q water and used as diffusion bottles, and 10 mL-weighing bottles were cleaned with acid and tied with a thin nylon cord as receiving bottles.

Diffusion and absorption methods are as follows. First, 2.00 mL of 0.10 mol/L H$_2$SO$_4$ was added into the receiving bottle. To the diffusion bottle the following reagents were added sequentially: water sample, 1.500 g of chemical reagent-Devardas Alloy, and 1.000 g of magnesia powder. Then, put the receiving bottle on the top of the diffusion bottle quickly and carefully, tied the thin nylon cord to the diffusion bottleneck, and closed the diffusion bottlecap. Finally, sealed the bottle cap with parafilm and placed the bottle into the thermostat bath oscillator. By reacting with chemical reagent-Devardas Alloy and magnesia, the nitrate in water sample was reduced to ammonia, which was then diffused into the receiving bottle and absorbed by sulphuric acid solution to form (NH$_4$)$_2$SO$_4$.

2 Results and discussion

2.1 Ion exchange experiment

2.1.1 Ion-exchange resin

Adsorption experiments were done respectively using 201 × 7, 202 × 7 and Bio-Rad AG1-X8 anion exchange resins for self-made water samples containing 2.00 mg N/L of KNO$_3$. The experimental conditions were as follows: the height of resin bed was 2.50 cm; the volume of water passing through the column was 1000 mL; and the flow rate was maintained at 700–800 mL/hr. By measuring the NO$_3$$^-$ concentration in the effluent, the adsorption rate (R_a) can be calculated using the following Eq. (1):

$$R_a = \frac{m_0 - m_e}{m_0} \times 100\% \tag{1}$$

where, m_0 is the initial NO$_3$$^-$ concentration in the water sample, and m_e is the NO$_3$$^-$ concentration in the effluent.

The calculated adsorption rates for anion exchange resins Bio-Rad AG1-X8, 202 × 7 and 201 × 7 were 97%, 82% and 78%, respectively. The adsorption efficacy of resin Bio-Rad AG1-X8 for NO$_3$$^-$ was the highest. It was also found that for resin 201 × 7 or 202 × 7, the adsorption rate could not reach 90% even with increased resin bed height. Considering the generally low NO$_3$$^-$ concentration of surface water, we chose the best-adsorption Bio-Rad AG1-X8 resin for the following experiments.

2.1.2 Resin bed height

In order to determine a optimal height of resin bed, adsorption experiments were carried out with different resin bed heights at different NO$_3$$^-$ concentrations. The volume of water passing through the column was 1000 mL, and the flow rate was 700–800 mL/hr. The experimental results are shown in Table 1.

As shown in Table 1, the exchange columns with the resin bed height 2.00, 2.50 to 3.00 cm have good adsorption efficacy for NO$_3$$^-$, and the adsorption rate generally can reach 95%. The adsorption rate increases with increasing resin bed height and the NO$_3$$^-$ concentration of water

Table 1   Comparison of adsorption efficacy for different resin bed heights

Resin bed height (cm)	NO$_3^-$ (mg N/L)		Adsorption rate (%)
	m_0	m_e	
2.00	0.500	0.030	94.0
	1.000	0.042	95.8
	2.000	0.050	97.5
2.50	0.500	0.015	97.0
	1.000	0.022	97.8
	2.000	0.026	98.7
3.00	0.500	0.012	97.6
	1.000	0.017	98.3
	2.000	0.019	99.1

m_0: initial NO$_3^-$ concentration in water samples;
m_e: NO$_3^-$ concentration in effluent.

sample. Considering the adsorption efficacy, experimental costs and time, we chose the resin bed height of 2.50 cm for the following experiments.

2.1.3 Flow rate of adsorption solution

The flow rate of the adsorption solution is one of the major factors affecting the adsorption efficacy. Experiments were conducted with 2.50 cm resin bed height of Bio-Rad AG1-X8 exchange column for the samples (KNO$_3$ concentration was 1.00 mg/L). At flow rates 600, 900, and 1200 mL/hr, the measured adsorption rates were 98.3%, 97.2%, and 95.3%, respectively. The results show that the flow rate has no significant impact on the adsorption efficacy. Considering the low concentration of NO$_3^-$ in watershed surface water and experimental time, we chose a flow rate of 700–800 mL/hr for the experiments.

2.1.4 Eluant

Bio-Rad AG1-X8 anion exchange resin shows a good adsorption-exchange capacity. Its relative selectivities for different anions are shown in Table 2.

Table 2   Relative choice between AG1-X8 resin and different anion*

Anion	I$^-$	HSO$_4^-$	NO$_3^-$	Cl$^-$	HCO$_3^-$
Relative selectivity	175	85	65	22	6

* Data sources: AG1, AG MP-1 and AG 2 Strong Anion Exchange Resin Instruction Manual.

It can be seen from Table 2 that Bio-Rad AG1-X8 anion exchange resin has the highest selectivity for iodide and the lower selectivity for bicarbonate. Therefore, we first tried 1.00 mol/L KI solution as eluant to perform the elution experiment, but found that the iodide was readily to be oxidized and unfit to be an eluant. Considering that HSO$_4^-$ might affect the following diffusion experiment and NO$_3^-$ was the target compound from sample, we chose chloride solution as eluant.

2.1.5 Flow rate of eluant solution

The resin column previously used for the adsorption experiment was used for testing the flow rate of eluant solution. KCl solution (2.00 mol/L) was used as the eluant. Each column was eluted with 40.00 mL eluant for 8 times, i.e., each time 5 mL. The next elution would not start until the previous eluant had completely flowed out. The

eluate was collected and its NO$_3^-$-N concentration was determined. The elution rate (R_e) can be calculated by Eq. (2).

$$R_e = \frac{m_e \times V_e}{(m_0 - m_t) \times V_0} \times 100\% \qquad (2)$$

where, m_e and V_e respectively denote the NO$_3^-$-N concentration and volume of the eluate, m_0 and V_0 respectively denote the NO$_3^-$-N concentration and volume of the self-made water sample, m_t is the remaining NO$_3^-$-N concentration in the effluent collected from adsorption experiment of water sample.

The calculated elution rates under elution flow rates 4.0, 8.0, and 12.0 mL/hr were 99.6%, 98.8% and 94.2%, respectively. The results show that the elution flow rate has an obvious influence on the elution rate. Especially as the flow rate is greater than 8.0 mL/hr, the elution rate decreases from 98.8% to 94.2%. Therefore, a strict control on the flow rate is needed. Taking into account comprehensively the elution efficacy and experimental time, we chose the flow rate of 6.0–8.0 mL/hr for our experiment.

2.1.6 Experiment for field sample

The field water sample was collected from Tianbao Watershed of Jiulong River, Fujian Province, China. The water sample, after vacuum-filtered, was loaded onto the anion exchange column, the solution was kept above the resin, and the flow rate was controlled at 700–800 mL/hr. Then 40.0 mL of 2.00 mol/L KCl solution was used to carry on elution at a flow rate of 6.00–8.00 mL/hr, and the adsorption rate and elution rate were determined during the experiment.

The experimental results showed that although the adsorption rates of NO$_3^-$ in field sample were all greater than 95%, the elution rates were between 70%–90%, obviously less than those of self-made water sample. Due to the complex composition of the surface water in watershed, the interfering materials might not be removed by vacuum-filtration from the field sample. Considering that the low elution rate could not meet the requirements for isotope measurement, we added an additional purifying procedure before the column exchange.

2.1.7 Modified ion exchange experiment

According to literature (Chen and Zhang, 1995; Xi et al., 1998), the Al$_2$O$_3$ chromatographic column can effectively remove the interfering matter from the water sample. Hence it was the first choice for our purifying column experiments. We attempted to add an Al$_2$O$_3$ chromatographic column before the anion exchange. For the preparation method of Al$_2$O$_3$ column, see Section 1.1.

Took 3000 mL the same vacuum-filtered field sample with NO$_3^-$ concentration of 1.21 mg N/L, and divided it into three aliquots: one went through the anion exchange column directly, without passing through Al$_2$O$_3$ chromatographic column (S1-ex); and the other two went through the Al$_2$O$_3$ column first at a flow rate of 1000–1500 mL/hr and then through the anion exchange column, respectively (S2-ex and S3-ex). After that, samples S1-ex and S2-ex
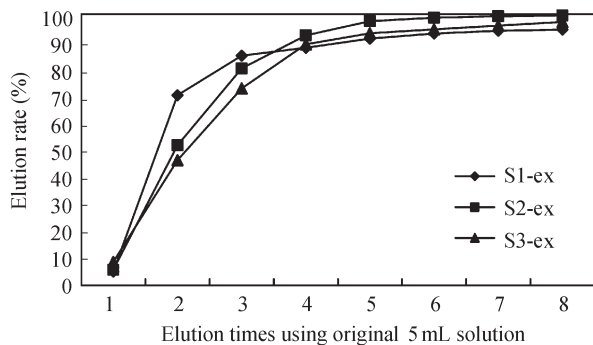
Fig. 1 Elution rate curve. Sample 1 and 2 were eluted with 2.00 mol/L KCl solution, sample 3 was eluted with 3.00 mol/L HCl solution.

Fig. 2 Recovery coefficient of NO_3^- at room temperature (RT) and 50°C.

were eluted respectively with 40.00 mL of 2.00 mol/L KCl solution (5 mL each for 8 times); sample S3-ex was eluted with 40.00 mL of 3.00 mol/L HCl solution (5 mL each for 8 times). The elution efficacy is shown in Fig. 1.

It can be seen from Fig. 1 that the NO_3^- elution efficacy is improved greatly by adding an Al_2O_3 column. It increases the elution rate to 95% or more, and for most of the field samples, it can effectively separate and enrich NO_3^- to meet the requirement for isotope measurement. In addition, both KCl and HCl solutions used as the eluant have good elution effect, while the KCl solution has slightly better efficacy than the HCl solution.

2.2 Diffusion-adsorption experiment

In order to test the diffusion-adsorption effects under different experimental conditions for water samples with same NO_3^--N concentration but in different medium, we prepared three water samples respectively: S1-da, prepared with Milli-Q water and KNO_3 (600 mgN/L of KNO_3); S2-da, prepared with Milli-Q water and KNO_3 (600 mgN/L of KNO_3), but also containing SO_4^{2-} (1000 mg/L) and Cl^- (1000 mg/L); S3-da, prepared with the field water sample and KNO_3, i.e., the surface water collected from Tianbao watershed of Jiulong River, was vacuum-filtered and pretreated by passing through the Al_2O_3 column and anion exchange column to remove the original NO_3^-. Then KNO_3 was added to sample S3-da to a certain NO_3^- concentration of 600 mgN/L. An aliquot of 80.0 mL of each sample was taken and equally divided into two fractions for the oscillation diffusion absorption experiment (see Section 1.2) at the room temperature and at 50°C, respectively. The diffusion-absorption time was 2, 4, 6, 8, and 10 days. The recovery coefficient (R_C) of NO_3^- can be calculated using Eq. (3), and the results are shown in Fig. 2.

$$R_C = \frac{m_0 - m_t}{m_0} \times 100\% \tag{3}$$

where, m_0 is the NO_3^- concentration of the solution prior to the diffusion, m_t is the remaining NO_3^- concentration of the solution after diffusion.

Figure 2 shows that the recovery increases with increasing diffusion time for both room temperature and 50°C. However, the diffusion-adsorption efficacy is poor at room temperature, even with the diffusion time of 10 days (the recovery is less than 80%). At 50°C the recovery increases
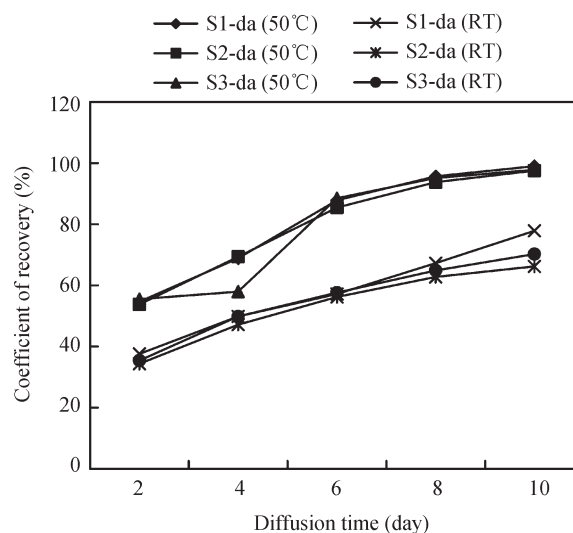
significantly. Even for the self-made and field samples with complex medium, the recovery can be more than 98% for the diffusion time of 10 days. It means that the diffusion absorption experiment with 50°C of temperature and 10 days of diffusion time can almost quantitatively recover all NO_3^- from the field sample.

2.3 Anion exchange-diffusion ^{15}N isotope fractionation experiment

In order to investigate whether the ^{15}N isotope was fractionated when carrying on the anion exchange and diffusion experiments, four aliquots of standard nitrate solutions (δ^{15}N value 7.50‰, every copy $m_N > 2.5$ mg) were respectively diluted to 1000 mL, and pretreated by the established methods described in Section 2.1, i.e., vacuum-filtered, passing through Al_2O_3 column and anion exchange column, and eluted with KCl solution. The obtained nitrate solutions were then put into the oscillation diffusion-absorption device for 10 days at 50°C (for the diffusion-adsorption experiment, Section 2.2). Finally the collected $(NH_4)_2SO_4$ solutions were sealed and sent to the Institute of Soil Science, Chinese Academy of Sciences for the δ^{15}N analysis. The measured δ^{15}N value for the 4 parallel samples were respectively 7.328‰, 7.900‰, 7.441‰, and 7.225‰ with average 7.473‰ and low standard deviation ($\sigma = 0.298$), suggesting a good overall precision and accuracy.

Previous studies by Xiao and Liu (2002) clearly showed that no significant fractionation would occur during the KNO_3 diffusion-absorption at 50°C for 10 days. The above experimental results also show that the improved anion exchange coupled to diffusion would not cause the ^{15}N isotope fractionation, further supporting that the pretreatment methods established in this study for ^{15}N isotope tracing analysis of surface water are practical and feasible.

3 Conclusions

Comprehensive analysis and assessment on the NO_3^- adsorption and elution efficacy were made through system-

atic simulation and comparative experiments for various conditions. Based on the experiments, a feasible pretreatment method was established for NO_3^- ^{15}N isotope tracing analysis of surface water. The results suggest that the use of Al_2O_3 column coupled with the Bio-Rad AG1-X8 anion exchange column in the chloride form, and KCl solution as the eluant, can effectively separate and enrich the NO_3^- from the water sample.

The improved anion exchange-diffusion method can separate and enrich NO_3^- from the watershed surface water with good recovery, and causes no significant ^{15}N isotope fractionation, satisfying the requirements for precise $\delta^{15}N$ isotope analysis.

In brief, the pretreatment method established by this study is not only simple in principle and easy to operate, but also less costly. It can efficiently separate and enrich NO_3^- from the watershed surface water, and meet the requirement of $\delta^{15}N$ mass spectrometric analysis. The results will be helpful to widen the applications of ^{15}N isotope tracing technique in the research on non-point source pollution in watershed.

Acknowledgments

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