

Determination of cosmogenic ^{32}P and ^{33}P in environmental samples

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Received 9 March 2012; accepted 14 May 2012

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Abstract

Understanding phosphorus dynamics in marine environment is of great importance, and appropriate tracers for phosphorus cycling in oceans are invaluable. In this study, two methods were developed for extraction, purification, and determination of naturally occurring ^{32}P and ^{33}P in rainwater, marine plankton and sediments using both a low-level beta counter (LBC) and an ultra-low-level liquid scintillation spectrometer (LSS). Blanks, chemical yields and counting efficiencies were quantified for both methods. The chemical purification of ^{32}P and ^{33}P separated by both procedures was validated by their decay curves. The absorber thickness of aluminum for LBC was assessed as 39.2 mg/cm². ^{32}P and ^{33}P specific activities in some rain samples were determined by both methods and showed good consistent results. The advantage of the LSS over the LBC is apparent in its high counting efficiency and in determining samples with high concentration of stable phosphorus. However, when measuring environmental samples with low concentration of stable phosphorus, such as rainwater, both methods can be used and each has its distinct advantage.

Key words: ^{32}P , ^{33}P , rainwater, plankton, sediments

Citation: Chen Min, Yang Zhi, Zhang Lei, Qiu Yusheng, Ma Qiang, Huang Yipu. 2013. Determination of cosmogenic ^{32}P and ^{33}P in environmental samples. Acta Oceanologica Sinica, 32(6): 18–25, doi: 10.1007/s13131-013-0305-5

1 Introduction

Phosphorus (P) is an essential nutrient for all living organisms, and controls many biogeochemical processes occurring in the biosphere. In marine environments, several studies suggest that phosphorus may limit biological production and nitrogen fixation in certain regimes (Benitez-Nelson and Karl, 2002; Planavsky et al., 2010). Phosphorus plays significant role in marine carbon cycling, and thereby, in atmospheric CO₂ dynamics (Benitez-Nelson and Buesseler, 1999).

As compared with carbon or nitrogen, biogeochemistry of phosphorus is poorly understood. Most previous studies were confined to the concentration and distribution of phosphorus, providing only limited information on how P affects biological production or carbon export (Benitez-Nelson, 2000; Pan et al., 2003; Dai et al., 2007; Qi et al., 2011). Understanding P dynamics in marine environment is of great importance. However, these studies have been hampered by methodology. The appropriate tracers for P cycling in oceans are invaluable.

Twin naturally occurring cosmogenic radionuclides, ^{32}P and ^{33}P , provide tracers for studying biogeochemistry of phosphorus in the upper ocean. Both ^{32}P ($T_{1/2}=14.28$ d, $E_{\text{max}}=1.71$ MeV) and ^{33}P ($T_{1/2}=25.3$ d, $E_{\text{max}}=0.249$ MeV) are produced primarily by cosmic ray interactions with atmospheric argon. Once produced, they are rapidly scavenged onto aerosols and enter the biosphere predominantly by wet depositions. Because the production rates of ^{32}P and ^{33}P are nearly independent of latitude in the troposphere and they have similar chemical characteristics as well as short half-lives, determination of ^{32}P and ^{33}P concentrations and $^{33}\text{P}/^{32}\text{P}$ ratios in wet deposition provides useful information on the transportation of air masses, penetration of stratosphere/troposphere and the residence

times of aerosols (Lal et al., 1960; Rama and Honda, 1961; Walton and Fried, 1962; Luyanas et al., 1970; Sanak et al., 1985; Waser and Bacon, 1994; Waser and Bacon, 1995; Benitez-Nelson and Buesseler, 1999). In addition to atmospheric production, an additional source of ^{32}P and ^{33}P is from spallation reactions on Cl, S, K and Ca in seawater during exposure to cosmic rays. However, this oceanic production has been estimated to be less than 5% for ^{32}P and 1% for ^{33}P of the local cosmogenic input that occurred at the Bermuda Atlantic Time-Series Station (Waser and Bacon, 1994). The previous studies show that $^{33}\text{P}/^{32}\text{P}$ ratios remain constant in rainwater (Waser and Bacon, 1995; Waser et al., 1996; Benitez-Nelson and Buesseler, 1999; Benitez-Nelson and Karl, 2002). After they enter marine environment, $^{33}\text{P}/^{32}\text{P}$ ratios vary due to the different decay rates of ^{32}P and ^{33}P . This can be used as an "internal clock" to estimate the residence times, uptake and regeneration rates and fluxes of import and export of P in various marine pools. Higher $^{33}\text{P}/^{32}\text{P}$ ratios suggest older net age of phosphorus in any particular reservoir of interest. Since the 1980s, ^{32}P and ^{33}P have been used as clocks to obtain the information on P cycling in rain, seawater, suspended particle and planktons (Lal et al., 1988; Waser et al., 1994; Waser and Bacon, 1995; Waser et al., 1996; Benitez-Nelson and Buesseler, 1999; Benitez-Nelson and Karl, 2002; Zhang et al., 2004).

In natural environments, concentrations of ^{32}P and ^{33}P are extremely low. The average activity concentrations in rainwater range from 0.2 to 6 dpm/dm³ (Lal et al., 1957; Walton and Fried, 1962; Waser and Bacon, 1995; Benitez-Nelson and Buesseler, 1998), whereas in seawater, dissolved ^{32}P and ^{33}P concentrations are 1 000 times lower (Benitez-Nelson and Buesseler, 1998). Hence, preconcentration of ^{32}P and ^{33}P from environ-

Foundation item: The National Natural Science Foundation of China under contract No. 41125020; a special scientific research project for public welfare supported by the State Oceanic Administration under contract No. 2010050012-3.

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mental samples is needed. For example, $\text{Fe}(\text{OH})_3$ coprecipitation was carried out to collect phosphorus from seawater (Lal et al., 1988). Lee et al. (1992) extracted dissolved inorganic phosphorus (DIP) using the selective adsorption of DIP-molybdenum blue complex onto a cation-form acrylic fiber and the extraction of total dissolved phosphorus by $\text{Fe}(\text{OH})_3$ -impregnated acrylic fiber proved very successful. The purification procedure for phosphorus was essentially similar to those used in the earlier studies by Lal et al. (1960), and involved a series of digestion, precipitation and ion-exchange steps. Stable phosphorus was served as a yield monitor in the procedure. In order to speed up the filtration and at the same time keep good extraction efficiency, Benitez-Nelson and Buesseler (1998) used $\text{Fe}(\text{OH})_3$ -impregnated filters to replace the acrylic fiber.

Two techniques were developed to determine the activities of ^{32}P and ^{33}P . One was by low-level β counter (LBC) in the form of $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$ precipitates (Lal et al., 1988; Lee et al., 1992; Waser et al., 1994). Since the maximum energies of beta particles from ^{32}P and ^{33}P are different, the low-energy beta emission from ^{33}P is easily blocked by the external absorber. Samples are counted twice by LBC: one with absorber to measure ^{32}P only, and then without absorber to obtain the total activity of ^{32}P and ^{33}P . The difference between both measurements represents the activity of ^{33}P . However, this technique was not suitable for measuring samples with high concentrations of stable P since the final counting source was thick and the self-absorption was serious (Waser et al., 1994). Recently, a new technique was developed for the measurement of ^{32}P and ^{33}P activities by ultra-low-level liquid scintillation spectrometer (LSS) (Benitez-Nelson and Buesseler, 1998). Activities of ^{32}P and ^{33}P were measured simultaneously with high efficiencies, and the concentration of stable P will not affect the detection.

In this study, the techniques for ^{32}P and ^{33}P determination in rainwater, plankton and sediments by LBC and LSS were developed. The detailed procedures for phosphorus collection, extraction, purification and measurement were described. Blanks, chemical yields, counting efficiencies and the ideal aluminum absorber thickness were quantified. ^{32}P and ^{33}P specific activities in rainwater were determined both by LBC and LSS technique and compared with each other.

2 Methods

2.1 Sample collection and pretreatment

2.1.1 Rainwater

During the period between December 2002 to April 2004, rainwater samples were continuously collected ($n=44$) at Xiamen University ($24^\circ 26.1317' \text{N}$, $118^\circ 05.4162' \text{E}$) at a height of 20 m. Five quadrat plastic tanks with total areas of 0.20 m^2 were used for rainwater collection. Soluble reactive phosphate (SRP) in random samples was measured. Our results show that the SRP concentrations (calculated by P) in rainwater varied from 0.006 to 0.024 mg/dm^3 during a small rainfall event of being less than 0.9 cm and lower than the detection limit during a large rainfall event of being greater than 1.9 cm (Table 1).

Considering the low phosphate concentrations in rainwater, 10 cm^3 of KH_2PO_4 (0.300 mg/cm^3 , calculated by P) was added to serve as a carrier and a yield monitor. Rain samples were acidified with HCl to pH ~ 2 , and equilibrated for 4–6 h. The sample solution was filtered through a Mixed Cellulose Ester (MCE) membrane with $10 \mu\text{m}$ pore-size in order to remove

large impurities. Sixty to ninety milligram of Fe (FeCl_3 solution) was added into the filtrate and left to equilibrate for 6–12 h. Ammonium hydroxide was added slowly to precipitate $\text{Fe}(\text{OH})_3$, which scavenges phosphorus. After standing for 24–48 h, decanting and the $\text{Fe}(\text{OH})_3$ precipitate was separated from the supernatant by centrifugation. The precipitate was washed with Milli-Q water. This step served to pre-concentrate P from a large volume waters and separate phosphorus from potassium, sodium and other major cation and anion ions. The precipitate was then dissolved in 1–2 cm^3 of concentrated HNO_3 to make 1 mol/dm^3 HNO_3 solution for further separation and purification of phosphorus.

Table 1. Phosphate concentrations in selected rainwater from Xiamen

Sampling date (y-m-d)	Rainfall/ cm	Phosphate concentration ^{1)/} $\text{mg}\cdot\text{dm}^{-3}$
2002-12-18	0.19	0.006
2002-12-19	2.73	b.d.
2002-12-27	1.97	b.d.
2003-01-07	2.80	b.d.
2003-03-10	5.40	b.d.
2002-04-01	0.12	0.013
2003-04-03	0.63	0.007
2004-03-03	0.85	0.024

Notes: ¹⁾ b.d. represents below detection limit.

2.1.2 Plankton

Plankton samples were collected by towing net ($100 \mu\text{m}$ mesh-size) in the northern Xiamen Bay around the Gulang Island (from $24^\circ 27' \text{N}$, $118^\circ 01' \text{E}$ to $24^\circ 29' \text{N}$, $118^\circ 03' \text{E}$). Microscopic observation indicates that the dominate species were *A. pacifica* and *Centropages tenuiremis*. Plankton samples were taken back to laboratory within half an hour and further concentrated by nets with $0.45 \mu\text{m}$ mesh-size, dried at $70\text{--}80^\circ \text{C}$ to obtain the dry weight. After combusted at $400\text{--}500^\circ \text{C}$ for 4 h, all of the phosphorus was converted to inorganic forms. Samples were repeatedly extracted with $10\text{--}20 \text{ cm}^3$ of concentrated HNO_3 in a Teflon pot by micro-wave digestion. The supernatant solution was transferred to a new Teflon beaker. The residue was rinsed with 1 mol/dm^3 HNO_3 solution three times, and the washing solution was combined into the digestive sample. The resulting solution was heated to dryness and re-dissolved in a 1 mol/dm^3 HNO_3 solution for further separation.

2.1.3 Sediments

Surface sediments with a depth of being less than 1 cm were collected every 4–8 m from the flood line during the ebb in the inter-tidal zone around the Xiamen Bay ($24^\circ 26' \text{N}$, $118^\circ 05' \text{E}$). Samples were then taken back to lab and weighed, dried at $70\text{--}80^\circ \text{C}$, and combusted at $400\text{--}500^\circ \text{C}$ for 4 h to convert all of the phosphorus to inorganic form. About 3–5 g of the combusted sediments was used for phosphorus extraction with the same procedure as the plankton samples. The digestive solution was converted to 1 mol/dm^3 HNO_3 solution for further separation.

2.2 Separation and purification of phosphorus

Procedures for the separation and purification of phosphorus were modified from Waser et al. (1994) and Benitez-Nelson and Buesseler (1998). Briefly, the resulting 1 mol/dm^3 HNO_3 solution was heated to $30\text{--}40^\circ \text{C}$ and excess ammonium molybdate (0.1 g/cm^3) was added. The sample solution was

stirred until a yellow precipitate of ammonium phosphomolybdate, $(\text{NH}_4)_3\text{PO}_4(\text{MoO}_3)_{12}$, appeared. This precipitation was used to reduce the amount of heavy metals, particularly Fe, Co, Ni, Cr, Ti and Zr (Waser et al., 1994). The heating temperature was set to below 50°C , otherwise molybdic acid may co-precipitate with Si, As and V, and disturb the further purification. If the samples had too many metal contaminations, the precipitate was re-dissolved in concentrated NH_4OH and acidified to $1 \text{ mol/dm}^3 \text{ HNO}_3$, and ammonium phosphomolybdate was precipitated a second time. After about 1 h, the precipitate was filtered through a MCE membrane ($0.22 \mu\text{m}$ pore-size, 47 mm in diameter), washed with $1 \text{ mol/dm}^3 \text{ HNO}_3$, and then dissolved in 1:3 (v/v) NH_4OH . The resulting solution was adjusted to $\text{pH} \sim 7$ with HCl and cooled in an ice bath. An excess reagent of $\text{MgCl}_2\text{-NH}_4\text{Cl}$ and then drops of concentrated NH_4OH were added during stirring. A white crystalline precipitate of ammonium magnesium phosphate ($\text{NH}_4\text{MgPO}_4\cdot 6\text{H}_2\text{O}$) was formed. While stirring was maintained, an excess of concentrated NH_4OH was added. The precipitate in solution was cooled in an ice bath for more than 30 min in order to ensure precipitation completely. This step was used to turn the precipitate into a form which can be dissolved in HCl for subsequent column separation.

The $\text{NH}_4\text{MgPO}_4\cdot 6\text{H}_2\text{O}$ precipitate was filtered through a MCE membrane ($0.22 \mu\text{m}$, 47 mm in diameter) and washed with 1:3 (volume/volume) NH_4OH solution. After dissolution in 50–80 ml of $9 \text{ mol/dm}^3 \text{ HCl}$, the resulting solution was loaded on a cation resin column (100–200 mesh, 8–10 cm high) to adsorb Ca, K, Fe, Al, V, W, Zn, Zr and Ti, which interfere with the final precipitation. The resin was subsequently washed with 20–30 ml $9 \text{ mol/dm}^3 \text{ HCl}$ to completely remove phosphorus. The effluent and rinses containing phosphorus were reduced to a small volume via evaporation on a hot plate. The pH was adjusted to 7 with concentrated NH_4OH , and diluted to 30–40 cm^3 with Milli-Q water. The resulting volume was recorded exactly for further calculation of phosphorus concentration. The following procedures were different depend on the counting technique used.

When ^{32}P and ^{33}P were measured by LBC, two subsamples of 0.3 cm^3 were removed from above solution for phosphorus determination. The remaining solution was put into an ice bath and precipitated again as described previously. The precipitate of $\text{NH}_4\text{MgPO}_4\cdot 6\text{H}_2\text{O}$ was collected onto a pre-weighted MCE membrane ($0.22 \mu\text{m}$ pore-size, 25 mm in diameter, the weight is W_m), and dried until the weight was constant. It was weighed again to obtain the weight of precipitate and membrane (W_{s+m}) for chemical yield's calculation. The membrane was sealed with tape (1.0 mg/cm^2 in thickness) and determined by low-level β counter.

When LSS was used for ^{32}P and ^{33}P measurements, the solution was taken to dryness, dissolved in 1 cm^3 Milli-Q water, and transferred to a pre-weighed liquid scintillation vial (the weight of the empty vial was W_{st}). Two cubic centimeter of Milli-Q water was used to rinse the container twice and combined into the vial. The vial with solutions was then re-weighed (W_{s+st}^1). Two subsamples of 0.015 cm^3 were removed for phosphorus determination using classic molybdenum blue method (Parsons et al., 1984). The vial containing sample solution was weighed again (W_{s+st}^2) in order to calculate the chemical yield. Five cubic centimeter of the cocktails (Optiphase HiSafe 3) was then added, and stored in the dark for more than 10–12 h prior to LSS counting.

2.3 ^{32}P and ^{33}P measurements

2.3.1 LBC

Both ^{32}P and ^{33}P are β -emitters, but their maximum beta energies differ ($E_{\text{max}}=1.71 \text{ MeV}$ for ^{32}P , $E_{\text{max}}=0.249 \text{ MeV}$ for ^{33}P). In this study, $\text{NH}_4\text{MgPO}_4\cdot 6\text{H}_2\text{O}$ precipitates were counted twice by LBC (Model BH1227, Beijing Nuclear Instrument Factory): once with aluminum absorber to measure ^{32}P only, and then without absorber to obtain the total activity of ^{32}P and ^{33}P . The difference between both measurements represents the activity of ^{33}P . The best thickness of aluminum foils for separating the β emissions of ^{32}P from that of ^{33}P was ascertained by experiments.

2.3.2 LSS

^{32}P and ^{33}P were determined simultaneously by Wallac 1220 Quantulus (Perkin Elmer Co) under the high-energy (^{14}C) mode. For most of β emitters ($E_{\text{max}} > 156 \text{ keV}$), the counting efficiency is usually higher than 95% under this mode. Quench will cause the ^{33}P and ^{32}P peaks to shift to lower energies outside the chosen energy regions of interest. In order to compensate for quench effects, the regions of interest should be adjusted by using the software of window auto-regulating under Wallac Easy View. Most of the quench was caused by residual HNO_3 in the sample, which can be avoided by carefully treatment.

2.4 Calculation of the chemical yields

For samples measured by LSS, phosphorus content (M_{LSS}) after the procedure is expressed by the following equation:

$$M_{\text{LSS}} = \frac{2m \times (W_{s+st}^1 - W_{st})}{W_{s+st}^1 - W_{s+st}^2}, \quad (1)$$

where m is the phosphorus content in the subsample determined by molybdenum blue method (mg P). W_{st} , W_{s+st}^1 , W_{s+st}^2 represent the weight of the empty vial, the weight of the vial containing sample solution, and the weight of the vial containing sample solution after removing subsamples for phosphorus determination, respectively. The chemical yield for LSS method (η_{LSS}) is given by:

$$\eta_{\text{LSS}} = \frac{M_{\text{LSS}}}{I} \times 100\%, \quad (2)$$

where I represents the added stable phosphorus (mg P).

For samples measured by LBC, phosphorus content (M_{BC}) is expressed as:

$$M_{\text{BC}} = \frac{m \times V_1}{V_2}, \quad (3)$$

where m is phosphorus content in the subsample (mg P), V_1 represents the solution volume prepared for counting source (cm^3), V_2 represents the volume used for stable phosphorus determination (cm^3). The chemical yield is calculated by two methods. One is based on the change of phosphorus content during the procedure, and expressed as:

$$\eta_{\text{BP}} = \frac{M_{\text{BC}}}{I} \times 100\%, \quad (4)$$

where η_{BP} represents the chemical yield derived from the content of stable phosphorus, M_{BC} is calculated by Eq. (3), and I is the added stable phosphorus (mg P).

The other method for estimating chemical yield is based on the weight change of $\text{NH}_4\text{MgPO}_4\cdot 6\text{H}_2\text{O}$ precipitates during the procedure, and expressed as:

$$\eta_{\text{BW}} = \frac{(W_{s+m} - W_m) \times 10^3 \times M_p}{M_{\text{NH}_4\text{MgPO}_4\cdot 6\text{H}_2\text{O}} \times I} \times 100\%, \quad (5)$$

where M_p and $M_{\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}}$ are molecular weight of stable phosphorus and $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$, respectively. W_{s+m} is weight of the membrane containing precipitates (mg), and W_m is the weight of membrane (mg).

2.5 Blank determination

To determine the blanks of the procedures, 10 cm³ of KH_2PO_4 solution (0.300 mg/cm³) was used to prepare the counting source based on the above procedures. Five blank sources were determined by LBC, and nine were determined by LSS. The difference between the blank sources and the inherent backgrounds of the instruments was used to calculate the net blank counting rates.

2.6 Determination of the counting efficiency

Counting efficiencies of LSS and LBC for ³²P and ³³P were determined by counting standards with known activities of ⁴⁰K ($E_{\text{max}}=1.32$ MeV) and ¹⁴⁷Pm ($E_{\text{max}}=0.224$ MeV), which have maximum beta energies close to those of ³²P ($E_{\text{max}}=1.71$ MeV) and ³³P ($E_{\text{max}}=0.249$ MeV).

For the determination of the counting efficiency by LSS, 0.117 0 g of KCl (14.75 Bq/g) was put into a counting vial, dissolved in 3 cm³ of Milli-Q water. Five cubic centimeter of Optiphase HiSafe 3 cocktails were added, and stored in the dark for more than 6 h. ⁴⁰K activities were measured by LSS. Similarly, a known activity of ¹⁴⁷Pm standard was counted by LSS for the determination of ³³P counting efficiency (Benitez-Nelson and Buesseler, 1998).

For the determination of the counting efficiency by LBC, 0.040 cm³ of ³²P standard solution [³²P-Na₃PO₄, (837.7±2.6) Bq/cm³] were dried on a stainless planchet until the weight was constant. ³²P activity was measured by LBC with and without Al absorbers. ¹⁴⁷Pm was also used as the substitute for ³³P. A known activity of ¹⁴⁷Pm standard solution was dried on a stainless planchet until the weight was constant. ¹⁴⁷Pm activity was measured by LBC with and without Al absorbers.

2.7 Purity validation of ³²P and ³³P

To validate the purity of ³²P and ³³P in our counting sources, two rain samples are counted at different time to obtain the decay curves of ³²P and ³³P by both LSS and LBC. The estimated half-lives of ³²P and ³³P from the curves were compared with their reference values.

3 Results and discussion

3.1 Blanks

The count rates of blank sources and the inherent backgrounds of both instruments were given in Table 2. For LSS, the averaged counting rate of the nine blank sources was (2.28±0.03) cpm, and the inherent background of the LSS was (1.97±0.08) cpm (Table 2). For LBC, the averaged counting rate of the five blank sources was (1.20±0.01) cpm, and the inherent background of the LBC was (1.17±0.03) cpm (Table 2). The above results indicate that the net blank of LSS was higher than that of LBC. However, it is compensated by high counting efficiency of the LSS.

Table 2. Net count rates of blank sources and inherent backgrounds of LBC and LSS

Sample No.	Net count rates by LBC/cpm		Sample No.	Net count rates by LSS/cpm	
	Blank sources	Instrument background		Blank sources	Instrument background
BC1	1.19±0.03	1.17±0.03	BS1	2.38±0.09	1.97±0.08
BC2	1.21±0.03		BS2	2.48±0.09	
BC3	1.21±0.03		BS3	2.16±0.09	
BC4	1.19±0.03		BS4	2.37±0.09	
BC5	1.20±0.03		BS5	2.26±0.06	
			BS6	2.26±0.06	
			BS7	2.31±0.14	
			BS8	2.14±0.13	
			BS9	2.16±0.14	
Average	1.20±0.01	1.17±0.03		2.28±0.03	1.97±0.08
Net blanks/cpm		0.03±0.03			0.31±0.09

The relative large variation of the blank sources by LSS was related to the counting mode. Benitez-Nelson and Buesseler (1998) suggested that ~32% of the blank counts of LSS was caused by the photons produced from the interaction between high energy cosmic radiation and cocktails, and ~68% was produced from the interaction between cosmic radiation and photoelectric amplifier or vial. In our study, the blank sources were determined every time before sample measurements in order to eliminate the instability of LSS blanks.

The net blanks of both procedures can be estimated by the counting efficiency (see Section 3.2) and the difference of the count rates between blank sources and inherent background of the instrument. The estimated net blanks of the procedures were 0.08 dpm and 0.32 dpm for LBC and LSS, respectively. These activities contributed only less than 7% to most of our measured environmental samples, including rainwater, plankton, and sediments. The low net blanks suggested that disturbance by reagents and vessels was ignored in our procedures.

3.2 Counting efficiency

The counting efficiencies for ³²P and ³³P by LSS were assessed as 92.5% and 92.3%, respectively (Table 3), indicating the LSS was suitable for low-energy β nuclides measurements, such as ³³P and ³H.

With and without Al absorbers, the counting efficiencies for ³²P by LBC were 31.8% and 32.6%, respectively (Table 3). The counting efficiency of ³²P was not reduced significantly by Al absorbers due to its high beta energy. In contrast with ³²P, the counting efficiency of ³³P was low and significantly affected by the Al absorbers. The counting efficiency of ³³P decreased from 6.1% to 1.2% with the Al absorber occurrence (Table 3). The low counting efficiency of ³³P induced a large uncertainty of ³³P activity. The pathways to reduce the counting errors included prolonging the counting times, increasing the sample volumes, or reducing the counting source thickness. Nevertheless, LBC was not suitable for ³³P determination in plankton samples since the high stable phosphorus would cause serious self-absorption of

β emission by a thick counting source. Waser et al. (1994) also determined the counting efficiencies of ^{32}P and ^{33}P by LBC as

25%–50% (^{32}P) and <20% (^{33}P), respectively. Obviously, the low counting efficiency of ^{33}P was the major disadvantage of LBC

Table 3. The counting efficiencies of ^{32}P and ^{33}P by LBC and LSS

Instrument	Counting efficiency /%			
	^{32}P		^{147}Pm	
	With Al-absorber	Without Al-absorber	With Al-absorber	Without Al-absorber
LBC	31.8±0.2	32.6±0.2	1.17±0.01	6.06±0.03
LSS	92.5±0.4		92.3±1.0	

compared with LSS.

3.3 Chemical yields

3.3.1 LBC

The chemical yields of phosphorus in 21 rain samples were estimated by two methods, i.e., the content change of stable phosphorus (η_{BP}) and the weight change of $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$ precipitate (η_{BW}). η_{BP} ranged from 41.9% to 95.9% with an average of 70.7%, while η_{BW} ranged from 39.4% to 98.5% with an average of 70.3% (Fig.1). For comparison, Benitez-Nelson and Buessler (1998) estimated η_{BP} as 31.6%–90.8% (averaging 68.6%) during their procedure. Lal et al. (1957) estimated η_{BW} as 50%–80%. Our chemical yields were consistent with these studies.

Our results show that the chemical yields obtained by the stable phosphorus (η_{BP}) were consistent with those obtained by the $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$ precipitate (η_{BW}), indicating both methods are suitable for chemical yield estimation during the separation and purification (Fig.1). In fact, both methods were used by researchers before, for example, Waser et al. (1996) used η_{BP} to calculate ^{32}P and ^{33}P activities in rainwater, suspended particles and planktons. Lal et al. (1957) used η_{BW} to calculate ^{32}P and ^{33}P activities in rainwater.

Although coincident chemical yields were obtained by both methods, each method has its disadvantage. η_{BP} obtained by the content change of stable phosphorus ignored phosphorus loss in the last step for $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$ precipitation. This

loss was evaluated as 1%–7% (Table 4), contributing a little to the whole chemical yields. η_{BW} represents the entire procedure, but it is difficult to accurately control the degree of $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$ drying. An excessive drying would result in an uneven surface of the counting source and a decrease of the counting efficiency, while an insufficient drying would result in the residual of crystal water in the precipitate and an overestimation of chemical yield.

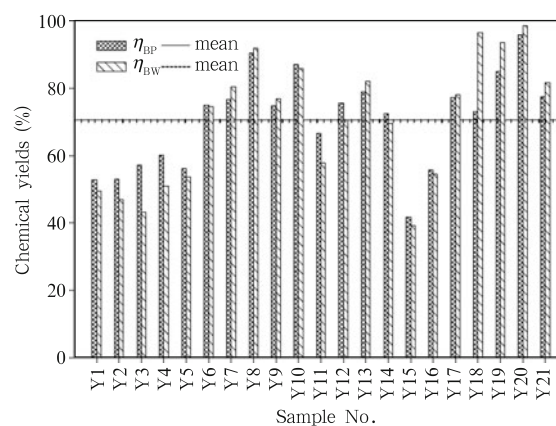


Fig.1. Chemical yields calculated from stable phosphorus (η_{BP}) and precipitate weight (η_{BW}) in rainwater samples.

Table 4. The chemical yield of each step in the procedure for phosphorus separation

Step	Chemical yield /%	
	Experiment I	Experiment II
Fe(OH) ₃ co-precipitation	91.0	89.0
Centrifugal	96.1	—
(NH ₄) ₃ PO ₄ (MoO ₃) ₁₂ precipitation	92.4	94.2
NH ₄ MgPO ₄ ·6H ₂ O precipitation	93.1	99.0
Dissolved in 9 mol/dm ³ HCl	100	99.9
Separation and purification by cation exchange column	99.2	99.0

In order to understand the phosphorus loss in each step in our procedure, the chemical yield of each step was determined by measuring the stable phosphorus in two rainwater samples. The chemical yields in all steps were greater than 92% except the Fe(OH)₃ precipitation (Table 4). The Fe(OH)₃ precipitation caused a major loss of phosphorus in our procedure (Table 4). The low chemical yield during Fe(OH)₃ precipitation was ascribed to the incomplete carry of phosphorus by Fe(OH)₃ precipitates from large volume waters and the adsorption of precipitates to the containers, just like that of thorium isotopes (Zhang et al., 2005). For comparison, Lal et al. (1988) reported an extraction efficiency of dissolved inorganic phosphate by

Fe(OH)₃ co-precipitation from large volume of seawater as being greater than 90%.

3.3.2 LSS

The chemical yields (η_{LSS}) of phosphorus for 26 plankton samples ranged from 46.7% to 97.0% with an average of 77.7% (Fig. 2), and those for 13 rainwater samples ranged from 34.8% to 95.5% with an average of 70.8% (Fig. 3). The yields for rain samples were slightly lower than those of plankton samples due to the Fe(OH)₃ co-precipitation. Using the averaged yields of rain and plankton samples, the chemical yield of phosphorus in the Fe(OH)₃ co-precipitation step was estimated as 91% (=70.8/77.7), coincided well with the above results in Table 4.

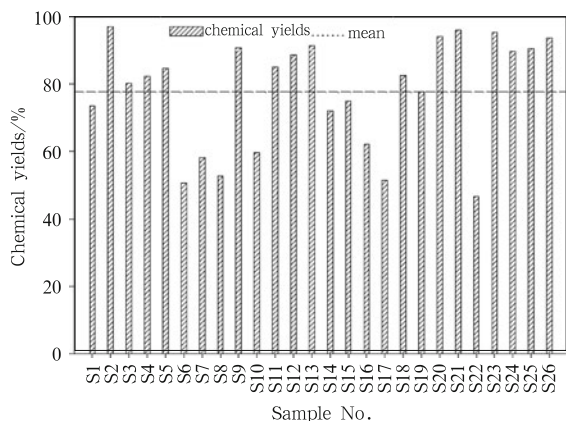


Fig.2. Chemical yields of phosphorus in plankton samples measured by LSS.

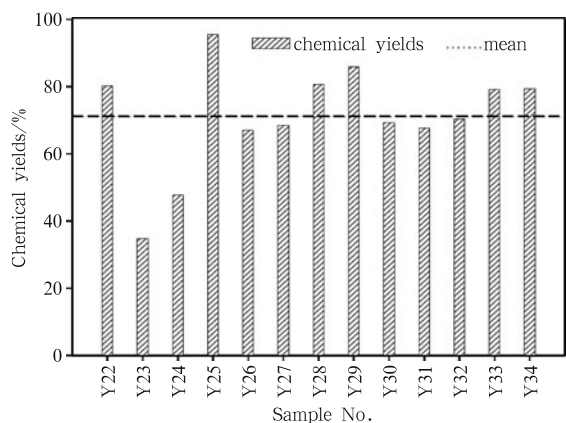


Fig.3. Chemical yields of phosphorus in rain samples measured by LSS.

3.4 The thickness of Al absorbers for ³²P and ³³P determination by LBC

Al absorbers were used to separate beta particles from ³²P and ³³P due to their large energy difference when measured by LBC. The transmission of electrons through a source was found experimentally well by the exponential absorption law for beta particles of a given maximum energy (Friedlander et al., 1981). The transmission of radiation (*I*) through the absorbing materials is given by:

$$I = I_0 e^{-\mu_m x}, \tag{6}$$

where *I*₀ is the intensity of a source with no external absorber, μ_m is the mass absorption coefficient (cm²/mg), and *x* is the absorber thickness (mg/cm²). The mass absorption coefficient is

related to the half-thickness (*x*_{1/2}) of an absorber as:

$$\mu_m = \frac{\ln 2}{x_{1/2}}. \tag{7}$$

The relationship between the net count rates of ³²P and ¹⁴⁷Pm (substitute for ³³P) and the Al absorber thickness is shown in Fig.4. The net count rates of ³²P decreased much slower than that of ¹⁴⁷Pm with the increasing Al thickness, which is related to the difference of their maximum energy of beta particles. Based on the absorption curves we obtained, the half-thickness of Al absorbers were estimated as 333 mg/cm² for ³²P and 14.7 mg/cm² for ¹⁴⁷Pm, respectively, indicating that a thin absorber would block most of the beta radiation from ¹⁴⁷Pm, but a few from ³²P. In this study, a thickness of 39.2 mg/cm² of aluminum foils was chosen to block the beta particles from ³³P. Under this condition, 85% of the beta particles from ¹⁴⁷Pm was block and only 8% for ³²P. The aluminum thickness used in our study was larger than that reported by Waser et al. (20 mg/cm², 1994), suggesting the different backgrounds between both beta counters.

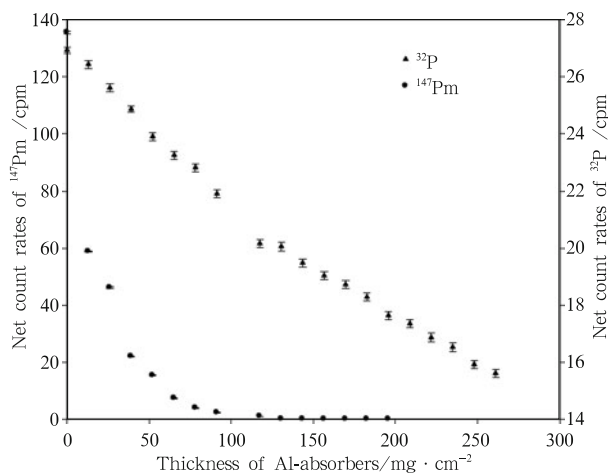


Fig.4. The relationship between the net count rates of ³²P and ¹⁴⁷Pm and the thickness of aluminum absorbers.

3.5 Errors

When ³²P and ³³P were measured by LBC, the samples were counted twice, and the activity of ³³P was calculated by the difference between both count rates. This would induce a large error for ³³P activity, especially for samples with low ³³P (Table 5).

Table 5. ³²P and ³³P specific activities in rain samples determined by LBC and LSS

Sample No.	Date (y-m-d)	Volume/dm ³	Yields /%	³³ P/dpm·dm ⁻³		³² P/dpm·dm ⁻³		³³ P/ ³² P) _{A.R.}	
				LBC	LSS	LBC	LSS	LBC	LSS
R1	2003-05-14	12.6	79.0	0.64±0.22	0.66±0.01	0.71±0.02	0.76±0.01	0.90±0.31	0.87±0.01
		45.6	81.9	—	0.09±0.00	—	0.11±0.00	—	0.82±0.04
R2	2003-06-11	29.6	22.6	—	0.20±0.01	—	0.25±0.01	—	0.78±0.07
		29.4	34.8	0.09±0.13	—	0.10±0.02	—	0.88±1.28	—

LSS has obvious advantages over LBC in ³²P and ³³P measurements. ³²P and ³³P were counted simultaneously with high efficiency by LSS. In addition, the peak spectrum obtained by LSS can be used to judge whether the sample was contaminated

by other beta emitters. As shown in Fig.5, ³²P and ³³P formed individual peak at different energy, indicating no other beta contamination in this rain sample. The overlap between both peaks was corrected by Cerenkov counting, that is, the sample was de-

terminated firstly without adding cocktails to obtain the Cerenkov radiation of ^{32}P , and these Cerenkov counts were used to correct ^{32}P counts after cocktail adding. Our results show that $\sim 7.5\%$ of ^{32}P activity was falling in the ^{33}P regions and $\sim 7.7\%$ of ^{33}P activity was falling in the ^{32}P regions.

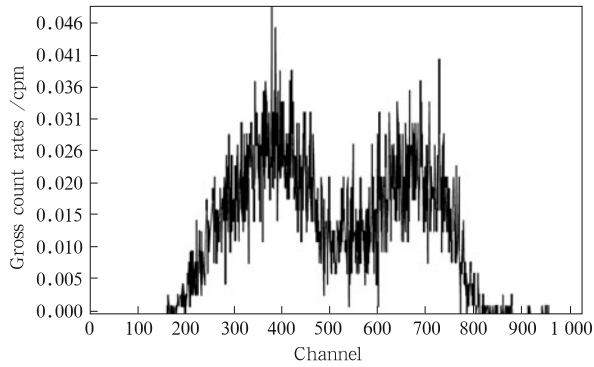


Fig. 5. The spectrum of ^{32}P and ^{33}P from a rain sample measured by LSS.

3.6 Purity validation

The net count rates with and without Al absorbers measured by LBC are shown in Fig.6 for a rain sample. With the Al absorbers, the half-life of ^{32}P calculated from the regression equation was (14.59 ± 0.37) d, well consistent with the reference value (14.28 d). Without the absorber, the half-life including ^{32}P and ^{33}P activities was estimated as (15.86 ± 0.73) d, fell into the range between ^{32}P (14.28 d) and ^{33}P (25.3 d).

The net count rates of ^{32}P and ^{33}P measured by LSS are

shown in Fig.7 for a rain sample. The estimated half-lives based on the decay curves were (16.90 ± 2.89) d and (27.72 ± 4.44) d for ^{32}P and ^{33}P , respectively, consistent with the reference values (14.28 d and 25.3 d for ^{32}P and ^{33}P respectively) within the uncertainty.

The above results suggest that our procedure was suitable for phosphorus separation and purification. The contamination by other radionuclides such as ^{210}Pb , and ^{210}Bi was eliminated by this procedure.

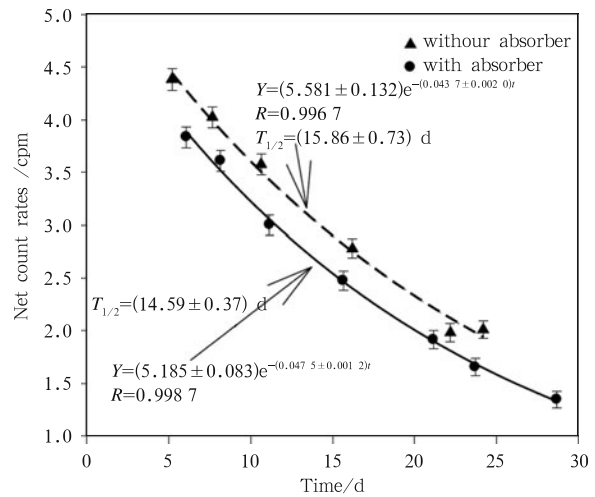


Fig. 6. The decay curves of ^{32}P and ^{33}P from a rain sample measured by LBC.

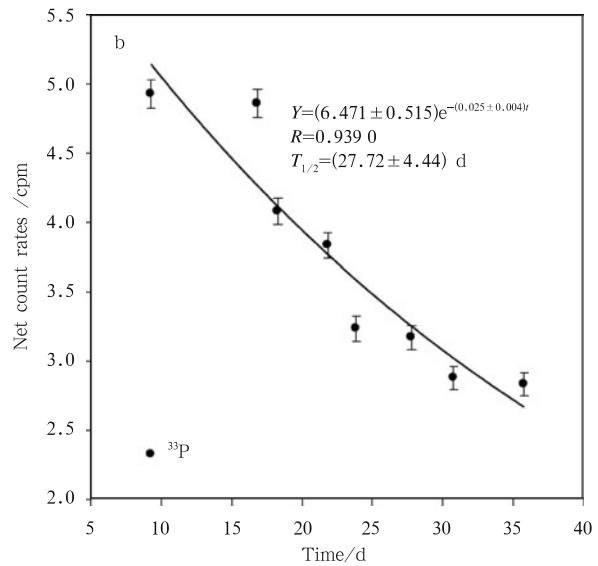
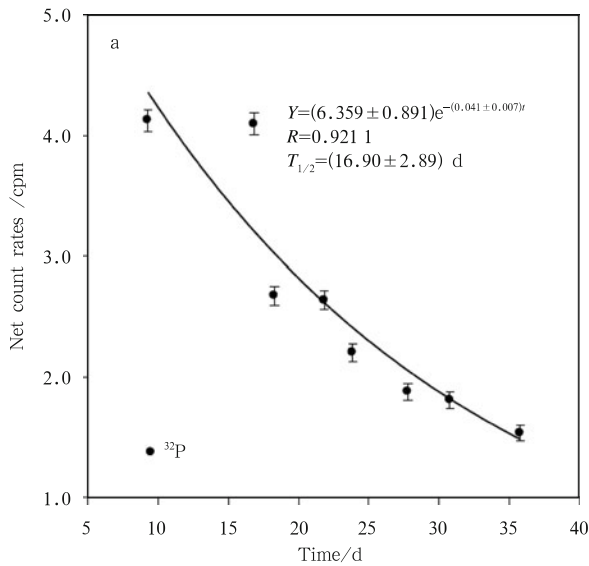


Fig. 7. The decay curves of ^{32}P and ^{33}P in a rain sample measured by LSS.

3.7 ^{32}P and ^{33}P activities in rainwater: comparison between LSS and LBC

^{32}P and ^{33}P in two rain samples were determined contemporarily by LSS and LBC, and used to evaluate both methods. The specific activities of ^{32}P and ^{33}P obtained by LSS and LBC methods were consistent within uncertainty (Table 5). For example, ^{32}P and ^{33}P specific activities for R1 sample

were (0.71 ± 0.02) dpm/dm³ and (0.64 ± 0.22) dpm/dm³ respectively obtained by LBC, while they were (0.76 ± 0.01) dpm/dm³ and (0.66 ± 0.01) dpm/dm³ respectively by LSS (Table 5). All of the ^{32}P and ^{33}P specific activities were within the previously reported range for rainwater (0.15–4.10 dpm/dm³ and 0.14–3.27 dpm/dm³ for ^{32}P and ^{33}P respectively) (Waser and Bacon, 1995; Benitez-Nelson and Buesseler, 1998; Benitez-Nelson and Bues-

seler, 1999; Benitez-Nelson and Karl, 2002). Although the data obtained by both methods were consistent, the counting errors by LBC were significantly larger than those by LSS.

4 Conclusions

Methods were developed for the collection, purification and measurements of natural occurring ^{32}P and ^{33}P by LSS and LBC. The advantage of LSS over LBC for ^{32}P and ^{33}P measurements was apparent in determining samples with high stable phosphorus. However, both methods were suitable for samples with low stable phosphorus, and each has its distinct advantage. The advantage of LBC method includes: (1) stability of blanks and less affected by environmental factors; (2) avoidance from the quench correction and the ascertainment of interest regions in analysis of spectrum. The advantage of LSS method includes: (1) high counting efficiency, and the resultant small error of counting; (2) accuracy of the chemical yield obtained by stable phosphorus measurement; (3) ^{32}P and ^{33}P were determined simultaneously; and (4) no influence by the amount of stable phosphorus. The methods developed here can be applied to environmental samples, including rainwater, seawater, suspended particles, plankton and sediments.

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