

Article

Stable Sulfur Isotope Fractionation by Anion Exchange Chromatography. Production of Compounds Enriched in ^{34}S

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A separação do isótopo ^{34}S foi obtida pela reação de troca isotópica entre solução de ácido sulfuroso e ânions bissulfito adsorvidos em resinas aniônicas tipo amônio quaternário (Dowex 1X8 e Dowex 2X8, malha 100-200) acondicionadas em seis colunas de resina de troca aniônica. Cada coluna de resina possuía 130 cm de comprimento com diâmetro de 2,2 cm. As colunas foram conectadas em série durante o deslocamento da banda de bissulfito. Para a experimentação, a banda de bissulfito fixada na resina aniônica, inicialmente na forma OH^- , foi eluída subsequentemente com 0,2, 0,3, 0,4 ou 0,6 mol L^{-1} HCl em solução. Ácido clorídrico em solução foi introduzido juntamente com nitrogênio sob pressão de 245 kPa, para impedir durante o deslocamento, a liberação de gases e a oxidação da banda de bissulfito. Os experimentos utilizando a resina Dowex 1X8 mostraram que os melhores resultados em termos de máximo enriquecimento (18,66 % em átomos de ^{34}S) e da massa total acumulada de ^{34}S (161,7 mg, excesso) foram obtidos eluindo a banda de bissulfito por 50 m com HCl 0,2 e 0,3 mol L^{-1} , respectivamente. A substituição da parte empobrecida da banda com bissulfito natural foi efetuada a cada deslocamento de 10 m.

The separation of the ^{34}S isotope was carried out by isotopic exchange between sulfurous acid in solution and bisulfite anions adsorbed on a quaternary ammonium (Dowex 1X8 and Dowex 2X8, 100-200 mesh) packed in six anion exchange columns. Each resin column packed with the anion exchange resin had a height of 115 cm and 2.2 cm in diameter. The columns were connected in series during the displacement of bisulfite bands. For the experiments, a band of bisulfite was fixed to the anion resin, initially in the hydroxyl ion form, and subsequently eluted with 0.2, 0.3, 0.4, or 0.6 mol L^{-1} HCl solution. The hydrochloric acid solution was kept under nitrogen at 245 kPa, in order to prevent the evolution of gases and the oxidation of the bisulfite band. The experiments with the Dowex 1X8 resin showed that the best results in terms of maximum enrichment (18.66 atom % ^{34}S) and total ^{34}S accumulated mass (161.7 mg, excess) were obtained by elution of the bisulfite band for 50 m with 0.2 and 0.3 mol L^{-1} HCl, respectively. The depleted portion of the band was periodically replaced with natural bisulfite, at 10 m intervals during elution.

Keywords: *isotopic enrichment, stable isotope, enriched sulphur, ^{34}S*

Introduction

Sulfur is one of the essential elements for plant and animal life. In plants it has many functions besides being part of amino acids and proteins: in the form of SO_4^{2-} it

activates enzymes, as SH it is an active group in enzymes and coenzymes (lipoic acid, thiamine, biotin), in the process of photosynthesis it participates in chlorophyll synthe-

sis, CO₂ absorption, carboxylase activity, ribulose-2P, and the phosphorylation reaction, and it is essential in the N fixation process in legumes¹.

More studies are needed to more precisely define the importance of sulfur in plant nutrition, since little attention has been given to this matter up to now. Although reports about sulfur deficiency in cultivated plants around the world, and especially in Brazil are common, few studies on this nutrient have been carried out. There is an urgent need for research in this field².

The isotopic technique has proven to be very valuable for elucidating aspects related to sulfur as a plant nutrient, and also for evaluating its utilization by cultivated plants. The radioisotope ³⁵S has been used as a tracer in studies of the soil-plant system, due to its availability, relatively low cost, and non-sophisticated analytical technique. ³⁵S has a decay of β⁻ 0.167 Mev and a half-life of 88 days³. Currently, there is a tendency, at the international level, to encourage the use of non-radioactive techniques in isotopic tracer research, especially in field experiments.

The use of ³⁴S as a sulfur tracer allows for the development of experiments without time restraints. Because the experimental material is not exposed to radiation, special safety precautions are not needed. Hence, the use of the tracer technique with the ³⁴S stable isotope is an alternative for evaluations of sulfur dynamics in the soil-plant system. Recently, laboratories in the USA have produced compounds enriched with the ³⁴S stable isotope. Because of this and also due to progress in the analytical technique using mass spectrometry, the first studies on the ³⁴S stable isotope using marked compounds have been developed^{4,5}.

Although the studies using compounds enriched with ³⁴S are recent, the results obtained show tremendous potential and are a good alternative in studies using this important nutrient for plants, especially in long term projects.

It is fundamental when using the ³⁴S tracer in research projects that the method of isotopic separation and the production of labeled compounds in the isotope be dominated. It is feasible to supply Brazilian researchers in soil, environmental and biological sciences in general with this product at a reasonable price, one which is lower than the foreign product, and similar to that of the ³⁵S radioisotope.

The goal of this work is to study the method of ³⁴S enrichment by anion exchange chromatography in resin columns. There is little information in the literature on the technical and operational details of the resin column system for the enrichment of ³⁴S, nor much related to the simultaneous separation of the other stable sulfur isotopes and isotopic analysis. Hence, different experimental conditions have been evaluated (*e.g.* resin type, eluent, chemical concentration, and band displacement) in order to obtain ³⁴S enrichments up to of 20 atom %. Studies have also been carried out to obtain simultaneous separation of the other

less abundant stable sulfur isotopes (³³S and ³⁶S). After obtaining ³⁴SO_{2(aq)} in the isotopic enrichment columns, it is possible to prepare other inorganic compounds enriched with the isotope (NaH³⁴SO₃, Na₂³⁴SO₃, Na₂³⁴SO₄, (NH₄)₂³⁴SO₄).

Experimental

Resins

Anionic resins of the strong base divinylbenzene polystyrene type (Dowex 1X8 and 2X8, 100-200 mesh) with the following characteristics were used: polymerization with 8% divinylbenzene (% DVB); functional group CH₂N⁺(CH₃)₃ for Dowex 1X8 and CH₂N⁺(CH₃)₂ (C₂H₄OH) for Dowex 2X8; anionic exchange capacity in Cl⁻ of 3.2 mmol g⁻¹ (dry resin) and 1.4 mmol g⁻¹ (moist resin); resin diameter (100-200 mesh): 74-149 μm (U.S. standard).

Column system and apparatus

The enrichment system consisted of six columns of acrylic, 130 cm in length and having a 2.2 cm internal diameter, filled with 100-200 mesh anionic resin (Dowex 1X8 or Dowex 2X8), up to a height of 115 cm, in the form of Cl⁻. Each column had in lower section an acrylic cylindrical apparatus connected to the tube by a screw. On the base of this acrylic part two stainless steel screens, 50- and 300-mesh, and one 300-mesh propylene screen were attached in sequence. Above them, a neoprene O-ring, made of C 3002-7B, was placed to ensure sealing. In superior section of the column, where the chemical solutions were poured, a PVC piston with two neoprene rings was adapted. When moved upwards or downwards this PVC piston maintained the level of the solution in the column constant, at a few mm above the surface of the resin bed. The columns were connected in series with the aid of 1 mm internal-diameter polyvinyl chloride tubing.

The containers with sodium hydroxide, sodium bisulfite, and deionized water were placed 4 m above the columns, and the container with hydrochloric acid eluent solution was leveled with the columns. The flux of hydrochloric acid through the resin layers in the columns was effected with the aid of a system working under nitrogen pressure, at approximately 245 kPa. The entire system can be observed in Fig. 1.

For the isotopic analysis, a mass spectrometer model ATLAS-MAT CH-4 was used, having the following features: analyzer tube curvature radius of 120°; ionization by electronic impact; admission system with molecular flux; simple ion collector, Faraday-type cup; and scanning analysis system.

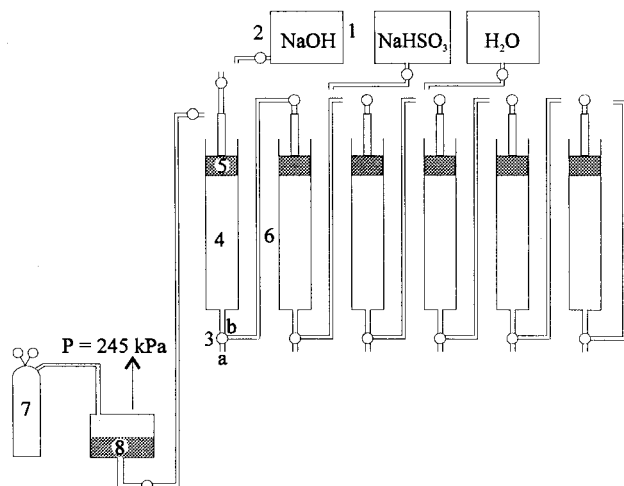


Figure 1. The column system for ^{34}S enrichment. 1. Solution containers; 2. Tap connection between the container and the column; 3a. Tap connection with waste system or counterflux tap connection with deionized water; 3b. Tap connection between columns; 4. Resin column; 5. Pressure piston; 6. Connection tubes; 7. Pre-purified nitrogen tank (high pressure); 8. Hydrochloric acid container.

Experimental development

During the development of this study the isotopic separation of ^{34}S was investigated using two types of resins and four concentrations of hydrochloric acid (0.2, 0.3, 0.4, and 0.6 mol L^{-1}) in five experiments (I to V), as shown in Table 1.

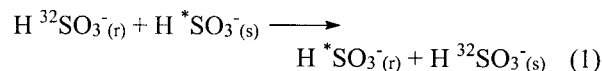
At the beginning of the experiments the resins were regenerated to the OH^- form with a solution of 2 mol L^{-1} sodium hydroxide, at a flow rate of $3.8 \text{ cm}^3 \text{ min}^{-1}$, followed by rinsing the resin layers in each column with deionized water, and fixing a band of sodium bisulfite solution in one of the columns (4 L of solution $0.15 \text{ mol L}^{-1} \text{ column}^{-1}$). This band was then eluted with an HCl solution, at a rate of $3.8 \text{ cm}^3 \text{ min}^{-1}$, having a second column connected in series. While the band displacement occurred, it was confined between two well-defined limits. These limits were made at front of the band by the equilibrium constants of the ionic exchange reactions between HSO_3^- in solution and adsorbed hydroxyl anions, and at the rear of the band by the reaction between Cl^- in solution and adsorbed HSO_3^- .

Table 1. Experimental conditions for the enrichment of ^{34}S in columns of anion resin (pressure 245 KPa).

Experiment (number)	Resin	HCl Concentration (mol L^{-1})
I	Dowex 2X8	0.3
II	Dowex 1X8	0.2
III	Dowex 1X8	0.3
IV	Dowex 1X8	0.4
V	Dowex 1X8	0.6

During displacement (elution), samples were collected along the band in order to evaluate the ^{34}S concentrations. The sampling procedure consisted of collecting small fractions of sulfurous acid at different positions along the band length, in one displacement column. The samples taken had equivalent amounts or twice as much sodium hydroxide as desired, making it possible to obtain sodium bisulfite or sodium sulfite. For the isotopic analysis by mass spectrometry, the samples were prepared by transforming the sodium bisulfite or sodium sulfite into sulfur dioxide under vacuum⁶. The region of the band with a normal isotopic concentration of ^{34}S (natural concentration threshold) was maintained at a minimum of 20% of the band. This control was done by the addition, when necessary, of a solution of 0.15 mol L^{-1} sodium bisulfite with ^{34}S natural concentration, following the discard of the same amount of band solution depleted of the isotope (with a concentration of less than 4.2 atom % ^{34}S).

In this process, the equilibrium between the sulfurous acid solution and HSO_3^- ions adsorbed to the anion exchange resin ($\text{HSO}_3^- - \text{H}_2\text{SO}_3$ system), for the fractionation of ^{33}S and ^{32}S , ^{34}S and ^{32}S , or ^{36}S and ^{32}S by ionic exchange in resin columns, is represented by Eq. 1⁷.



where *S represents the isotopes ^{33}S , ^{34}S , or ^{36}S , and (r) and (s) stand for resin and solution, respectively.

Results and Discussion

A summary of the main results is given in Table 2, showing that Experiments II and III had the best results. The data shown in Table 2 indicate that the best results for maximum enrichment ($18.66 \text{ atm}\% \text{ }^{34}\text{S}$) and total ^{34}S accumulated mass (161.7 mg , excess) were obtained by 50 m of elution of the bisulfite band with 0.2 and 0.3 mol L^{-1} acid, respectively. The experimental data proved that in experiments II and III the enriched band was established in the last 20 and 35 cm, respectively. These results, which can be observed in Fig. 2 and Table 2, show that a higher accumulation of ^{34}S mass occurs in Experiment III (161.7 mg), when compared to Experiment II. One of the most relevant parameters is the accumulation rate (excess) of the isotope ^{34}S (mg h^{-1}). The data in Table 2 indicate that this rate is approximately 20% higher in Experiment III (HCl 0.3 mol L^{-1}) when compared to Experiment II (HCl 0.2 mol L^{-1}).

Under the conditions used for experiments II and III, the enrichment curves for ^{34}S (Fig. 2) were obtained after 50 m of bisulfite band elution. It is possible to obtain from Experiments II and III, 6.46 and 7.37 mmol sulfurous acid respectively as a column effluent, which corresponds to 1 linear centimeter of the band (at the rear of the band). Table

Table 2. Parameters related to ^{34}S Enrichment Experiments by Ionic Exchange Chromatography (experiments I to V)

Exp. N°	Maximum Concentration* (atom %)			Average Concentration (atom %)			Accumulated ^{34}S mass (mg, excess)			Time** days	Rate mg h^{-1}
	10 ¹	30 ¹	50 ¹	10 ¹	30 ¹	50 ¹	10 ¹	30 ¹	50 ¹		
I	7.48	8.73	--	5.10	5.31	--	80.2	105.4	--	126	0.22
II	9.27	15.13	18.66	6.03	7.98	9.38	68.7	99.7	145.5	61	0.16
III	8.5	13.28	15.08	5.18	6.73	7.40	45.9	101.5	161.7	83	0.19
IV	7.34	--	--	5.35	--	--	25.3	--	--	--	0.15
V	5.45	--	--	4.69	--	--	7.5	--	--	--	0.04

* values estimated by the regression equation of the enriched profiles to the end point (0 cm) of the band; ** time necessary to obtain enrichment of 20 atom % ^{34}S on the limit of the back of the band.

rate: medium rate of accumulation (excess) of the ^{34}S isotope.

¹ distance (meters) of band elution.

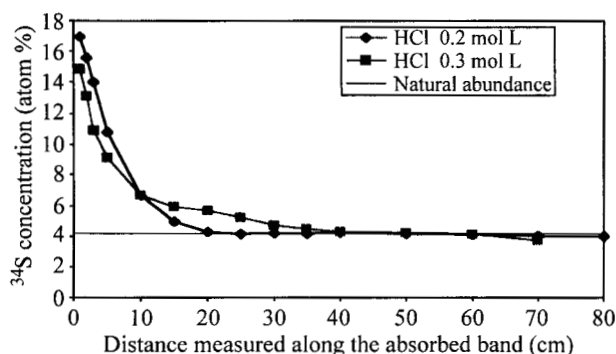


Figure 2. Profiles of an adsorbed bisulfite band (80 cm) after 50 m of elution.

2 also shows that Experiment V had the least favorable results in terms of ^{34}S isotope enrichment.

Analyzing Table 2, it can also be observed that Experiment I presented results comparable to Experiments II and III. However, the use of the resin Dowex 2X8 in this experiment was inadequate under these experimental conditions, especially due to difficulties in visualizing the band limits, causing isotopic mixtures. This made it impossible to proceed with the experiment with the distance of the band elution superior to 30 m. In Experiments IV and V the distance of the band elution was achieved only up to 10 m (Table 2), due to the concentrations of hydrochloric and sulfurous acid, which constantly caused corrosion of the stainless steel at the column bases. This caused many difficulties for the enrichment process including resin loss, discontinuance of the displacement band, isotopic mixtures, and chance of enrichment band loss, among others.

In Experiment II, the process had continuity (routine) after 50 m of band elution. It was possible to obtain for every 14 m from the last centimeter of band, an average of 6.79 mmol of sulfurous acid enriched at 16.76 atoms % in ^{34}S . This amount of sulfurous acid can be converted to 706, 855, or 964 mg of sodium bisulfite, sodium sulfite, or

sodium sulfate, respectively. Sodium sulfate can be obtained by the oxidation of the solution of sodium sulfite with oxygen.

After 50 meters of band elution the sulfurous acid solution was sampled at about 12 cm from the rear limit. This had the objective of evaluating the presence of sulfate which could originate from sulfite oxidation. The concentrations of sulfate and sulfite in solution were 0.43 and 35.88 mg cm^{-3} , respectively. These results indicate low oxidation, since about 1.2% of the initial sulfite was oxidized. There is also an indication that the system under nitrogen pressure in the reservoir of the HCl eluent solution was efficient in terms of preventing the dissolved air in solution (especially O_2) from favoring the oxidation reaction.

In Experiment II the isotopic concentration of the sulfur isotopes was also determined. The ratio of ^{33}S , ^{34}S and ^{36}S isotope concentrations at 1 centimeter from the back of the band limit to the natural abundance values were 2.0, 4.0, and 5.3, respectively. These values allow for the inference that the fractionation values (α) for the isotopes ^{33}S , ^{34}S , and ^{36}S in equilibrium with the most abundant sulfur isotope (^{32}S) should obey the following order: $\alpha^{36}\text{S} > \alpha^{34}\text{S} > \alpha^{33}\text{S}$.

Conclusions

Based on the results obtained it can be concluded that: a) the use of Dowex 2X8 resin for ^{34}S isotopic enrichment is not adequate under the experimental conditions used, due to the problem of visualization of the rear of the band; b) it is possible to obtain sulfurous acid with ^{34}S enrichment of 20 atom % in anionic resin columns of Dowex 1X8, by displacing the bisulfite band with HCl 0.2 or 0.3 mol L^{-1} eluent solution; c) by using the proposed system of resin columns, after 50 m of elution, 6.46 mmol (17.33 atom % of ^{34}S) and 7.37 mmol (16.93 atom % of ^{34}S) of sulfurous acid are obtained in the last linear centimeter of the band

with the use of 0.2 and 0.3 mol L⁻¹ HCl, respectively. The average rate of excess accumulation of ³⁴S is 0.16 and 0.19 mg per hour of elution with 0.2 and 0.3 mol L⁻¹ acid, respectively; d) in routine, after the first 50 m of band elution, under experimental conditions, it is possible to obtain 6.79 mmol of sulfurous acid for every 14 m of band elution with approximately 17 atom % in ³⁴S, in the last linear centimeter at the rear of the band; e) the resin column system, working at a pressure of 245 kPa under nitrogen atmosphere was able to control the release of gases and especially sulfite oxidation.

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