Research article

Preparation of ^{31}Si labelled silicate: a radiotracer for silicon studies in biosystems

Short title: Preparation of ^{31}Si tracer

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Summary

No-carrier added ^{31}Si was produced by $^{31}P(n,p)^{31}Si$ in the nuclear reactor of the Delft University of Technology. Several methods were investigated to remove the side product ^{32}P, and the chemical form of ^{31}Si was investigated. All methods except one gave good results. Anion exchange with Dowex resin gave the best results in activity concentration ($3.8 \cdot 10^6$ Bq/ml, with only $3.2 \cdot 10^3$ Bq/ml ^{32}P as by product) and specific activity ($A_s > 17$ TBq/g). The product is suitable for biological systems.

Key words: ^{31}Si; ^{32}P; no-carrier added; production

Introduction

Silicon (Si) is the second most abundant element (25.7 % w/w) in the earth's crust, being exceeded only by oxygen (49.2 %).[1] Its three stable isotopes, ^{28}Si, ^{29}Si and ^{30}Si, have natural abundances of 92.23, 4.67 and 3.10 %, respectively. Silicon is undoubtedly important for many living organisms and it may have played a significant role in the origin of life.[2] It is considered to be beneficial or essential to some plants, higher animals and humans, although only a few functions of silicon have been unravelled so far.[3,4] In biosystems and natural waters silicon is always in the chemical form of silicate (ortho, meta or higher polymerized). At low concentrations (< 5 mmol/l) it exists in the form of orthosilicate ($Si(OH)_4$), while above this concentration polymerization will take place to "metasilicate" (orthosilicate oligomers). At very high concentrations formation of silica gel ($(SiO_3^{2-})_n$) will occur. Metasilicate can depolymerize to orthosilicate when diluted to concentrations below 5 mmol/l.[5,6]

A convenient way for measuring transport rates in biosystems, including man, is by means of radioactive tracers that are chemically and physically identical to the compound/substance of interest. For silicon two radionuclides are suitable to be used as labels in such tracers, namely ^{31}Si ($t_{1/2}$ = 2.62 h, decays to ^{31}P (stable)) and ^{32}Si ($t_{1/2}$ = 160 y, decays to ^{32}P (radioactive)).[7] Both radionuclides emit β^- radiation, with maximum energies of 0.225 MeV (0.07%) and 1.49 MeV (99.9%) for ^{31}Si and of 0.213 MeV (100%) for ^{32}Si. In addition, ^{31}Si emits 1.27

MeV γ-rays (0.07%).[8,9,10] Instead of Si radionuclides, radioactive Ge (^{68}Ge or ^{71}Ge) has been used to study the behaviour of Si,[11,12] but this is probably not representative under all circumstances.

^{32}Si can be produced by either the ^{37}Cl(p,2pα)^{32}Si reaction or the reaction sequence ^{31}P(n,γ)^{32}P(n,p)^{32}Si using an accelerator or a high neutron flux nuclear reactor respectively.[13] ^{32}Si labelled compounds have been used for example in studies of silicon in marine food webs and for human and animal uptake kinetics.[14,15,16,17] However, the use of ^{31}Si is advantageous in short-term experiments, because (a) it is relatively easy to produce in high yields using a relatively low neutron flux reactor, (b) it does not decay to ^{32}P which can interfere with radioactivity measurements and (c) it results in far less radioactive waste. In addition, double tracer experiments using both ^{31}Si and ^{32}Si would become feasible.

^{31}Si can be produced by reactor thermal neutron irradiation of silicon or its compounds by the ^{30}Si(n,γ)^{31}Si nuclear reaction with a cross section (σ) of 0.108 barn. It can also be produced by fast neutron irradiation of phosphorus or its compounds by the ^{31}P(n,p)^{31}Si nuclear reaction having a threshold energy of 0.7328 MeV and a σ for fast neutrons of 0.034 barn.[7,8,17,18] Preparation of ^{31}Si from ^{31}P using the (n,p) route is accompanied by the simultaneous production of ^{32}P ($t_{1/2}$ = 14.26 d) due to the presence of thermal neutrons. The involved nuclear reaction ^{31}P(n,γ)^{32}P has a σ of 0.16 barn. ^{32}P emits β^- radiation with maximum energy of 1.710 MeV.[7,8] For doping experiments ^{31}Si is often produced by the ^{30}Si(d,p)^{31}Si reaction using an accelerator,[19,20] but no information is given on a purity check of the produced nuclide. We will focus on the production of ^{31}Si in a nuclear reactor. The physical data of the mentioned radionuclides are summarized in Table 1.

Our present study aims at producing ^{31}Si via the ^{31}P(n,p)^{31}Si reaction followed by radiochemical processing and chemical characterization of the final product for use as a label for biological studies with high specific activity. Note that silicon is present as a contaminant in water and in many chemicals, which influences the specific activity of the final product. In several studies ^{31}Si was produced by the ^{31}P(n,p)^{31}Si [21,22,23] reaction. However the specific activity was not given,[21] or the silicon concentration was not determined [21,22] which makes the found specific activity questionable. Also the chemical form of the tracer was not investigated.[21,22,23] As target material ^{31}P free of silicon impurities is preferred instead of ^{30}Si because of the possibility to produce no carrier added ^{31}Si, and because the natural abundance of the target material is 100%, so no enrichment is needed, in contrast to ^{30}Si which has a low abundance in natural Si, or is very expensive in enriched form. The use in biosystems implies the final radiotracer solution should have a pH that matches the system to study. It should have a chemical form identical to the natural non-radioactive form, and it should not contain toxic or harmful substances.

Results and Discussion

Production of the radionuclide

Phosphoric acid was irradiated as described in the experimental section. The ^{31}P(n,p) ^{31}Si and ^{31}P(n,γ) ^{32}P reactions resulted in the production of ^{31}Si and ^{32}P as determined using both a liquid scintillation counter (^{31}Si and ^{32}P) and a well-type Ge-Li-detector (^{31}Si). An amount of 4.3 ± 0.8 MBq ^{31}Si per mmol P was formed, accompanied by 5.7 ± 1.1 MBq ^{32}P under the conditions in the reactor. To determine the presence of radionuclide contaminations (apart from ^{31}Si and ^{32}P) half-live times were checked by mathematical fitting of the decay data (Figure 1). The found half-live values, 2.67 ± 0.05 hours and 13.98 ± 0.16 days, respectively, agreed well with the literature.[7,8] This indicates the absence of major radionuclide contaminations.

The ^{31}Si/^{32}P ratio's can be modified by the use of neutrons with different energies. In addition, shielding material with a high thermal neutron capture cross-section (e.g. cadmium or boron) during the irradiation can be used to reduce the occurrence of the ^{31}P(n,γ)^{32}P reaction and to increase the ^{31}Si/^{32}P ratio. However, this would slightly reduce the ^{31}Si yield and is only required when ^{32}P interferes with the experiments.

Removal of ^{32}P from the irradiated target material

Five methods to remove ^{32}P from the irradiated target material solution have been investigated: three using chemical precipitation (Method 1 – 3) and two using anion exchange (Method 4 and 5).

Purification of the irradiated material by chemical precipitation of phosphate (including ^{32}P) was carried out as described in the experimental section. Precipitation of ^{32}P with barium carbonate (Method 1) and silver acetate (Method 2) gave good results (Table 2). Removal of ^{32}P as barium hydrogen phosphate (Method 1) or silver phosphate (Method 2) was successful although 1.1 % (Method 1) or 0.36 % (Method 2) of it remained in solution, calculated as percentage of ^{32}P at the end of neutron bombardment. 73 % (Method 1) or 70 % (Method 2) of the initially present ^{31}Si remained in the purified solution, which make these procedures suitable for the production of ^{31}Si labelled silicate. The specific activity of ^{31}Si amounted to 4.8 ± 1.4 TBq/g (Method 1) or 3.0 ± 0.08 TBq/g (Method 2). Probably non-radioactive silicon was present as a contaminant in the chemicals used. Whether the removal of ^{32}P by Method 1 could be improved was investigated by means of centrifugation instead of filtration to remove small precipitate particles that can pass the filter, but no difference was found. Sequential precipitation with fresh barium carbonate or silver acetate did not lead to higher recoveries of ^{32}P. Precipitation with lanthanum nitrate (Method 3) removed more than 99.95% of the original ^{32}P as lanthanum phosphate. However, 99.5% of the ^{31}Si was co-precipitated, which clearly makes this method unsuitable for the preparation of the tracer (Table 2). X-ray diffraction analysis revealed the presence of phosphate in all precipitates from the first precipitation step of Method 1, 2 and 3. In case of Method 1 and 2 the original salt ($BaCO_3$ or Ag acetate) is also precipitated, probably as a result of pH change (Table 3). In Figure 2 a typical X-ray diffraction spectrum is shown.

Anion exchange was investigated as a method for phosphate removal from the irradiated target material. Silicic acid has its first pK value at 9.66 and phosphate at 2.12.[1] As long as the pH is kept between 3 and 9 silicic acid will not be dissociated and will pass through the resin whereas dissociated phosphate will interact with the anion exchange resin. Above pH 9 silicate will also start to dissociate and interact with the anion exchange resin. This principle has been investigated with Dowex anion exchange resin in laboratory made columns (Method 4) and with a commercially available prefab column (Method 5) as described in the experimental section.

Anion exchange purification at pH 5 resulted in 43.9 % recovery of ^{31}Si and 99.7 % removal of ^{32}P (Method 4), and in 91.0 % recovery of ^{31}Si and 99.6% removal of ^{32}P (Method 5). The obtained specific activity was 17.8 ± 0.4 TBq/g (Method 4) and 0.21 ± 0.02 TBq/g (Method 5). Solution refined by Method 5 contained a rather high amount of Si (5.14 ± 0.09 mg/l). The not purified solution contained 0.010 ± 0.001 mg/l Si, which indicates the column itself contains Si as a contaminant which is released during elution. Method 4 yielded a solution containing 0.21 ± 0.01 mg/l Si. Probably Si is removed during pretreatment with 8 M HNO_3. It is not possible to treat Method 5 columns with 8 M HNO_3, but probably the Si contamination can be reduced by increasing the volumes of NaCl solution and water during pretreatment. The ^{31}Si yield (Method 5) could be improved to 96.7% by rinsing the column with water pH 5 (volume equal to bed volume) after elution. It was not possible to remove the remaining ^{32}P by a second elution (Method 5). The results are summarized in Table 2. The influence of the pH has been investigated for pH 2-8 (Method 5). At pH 2 20% of ^{32}P remains in solution, above pH 4 99.7-99.9 % of ^{32}P is removed. Above pH 6 the recovery of ^{31}Si shows a declining tendency (Figure 3). This is an indication ^{31}Si has the silicate chemical form. In the next two experiments the chemical form of the tracer was further investigated.

It was not possible to completely remove ^{32}P by any method. The results of Method 3 indicate ^{32}P exists for at least 99.95% as orthophosphate. The precipitation product, lanthanum orthophosphate, has a very low solubility (K_s = $10^{-25.75}$) [24], so only minute amounts of orthophosphate can remain in solution. Possibly the remaining ^{32}P does not exist in phosphate form, but is chemically altered during irradiation.

The $^{31}P(n,p)^{31}Si$ reaction suggests the possibility of carrier free ^{31}Si preparation. But the results show all purified tracer solutions contain non-radioactive silicon, which is probably introduced as contaminants of the used chemicals. When the silicon content is kept low by the use of ultrapure chemicals high specific ^{31}Si activities can be obtained.

Investigation of the chemical form of ^{31}Si

When a radiotracer is used to investigate the role of silicon in biosystems it is important that the chemical form is identical to that of natural silicon. In natural waters and biosystems silicon is usually present in the form of silicate[5]. In dilute aqueous solutions (< 5 mmol/l) silicate molecules exist in the form of silicic acid, $Si(OH)_4$ or orthosilicate ($SiO(OH)_3^-$), and in higher concentrations as "metasilicate" (oligomers of orthosilicate). In highly concentrated solutions polymerization (gel formation) occurs. The gel formation process is started when a concentrated silicate solution (> 0.1 mol/l) is brought into contact with a strong acid.[5,6] This results in a gel phase with a silicate concentration higher than in the starting solution, and a water phase (supernatant) with lower silicate concentration than in the starting solution.

In this experiment the ability of silicate molecules to form a gel is used to investigate the chemical form of ^{31}Si. If ^{31}Si is in the silicate form it will participate in the chemical reaction of the polymerization process and will be incorporated in the gel matrix. As a result the ^{31}Si activity per ml gel (A_{gel}) will be higher than the activity per ml starting solution (A_0), and the activity per ml supernatant (A_{sup}) will be lower than A_0. In other words: $A_{gel}/A_0 > 1$ and $A_{sup}/A_0 < 1$. If ^{31}Si is not in the silicate form the activity will be equally distributed over gel and supernatant. The results can be compared with ^{32}P present in the tracer solution, which can not participate in the gel forming process. The activity of ^{32}P will be equally distributed over gel (trapped in the gel matrix) and supernatant ($A_{gel}/A_0 = A_{sup}/A_0 = 1$).

The experiment was conducted as described in the experimental section. The amount of gel that is formed depends on the final concentration of HCl (Figure 4). Activities of ^{31}Si and ^{32}P were determined before addition of HCl (to calculate A_0), and after gel formation (to calculate A_{gel} and A_{sup}), and the ratios A_{gel}/A_0 and A_{sup}/A_0 were calculated for both nuclides (Figure 5). The results show $A_{gel}/A_0 >1$ and $A_{sup}/A_0 < 1$ for ^{31}Si, especially when more acid is added. This indicates the participation of ^{31}Si in the gel forming process, indicating ^{31}Si is in the silicate form. For ^{32}P both A_{gel}/A_0 and A_{sup}/A_0 are about 1, resulting from an equal distribution of the nuclide over gel phase and supernatant.

To confirm the silicate form of ^{31}Si as suggested by the above experiment its behaviour is further investigated using paper chromatography. Silicic acid ($Si(OH)_4$) has a pK value of 9.66,[1] which means the molecules are non-dissociated at low and neutral pH. At pH 8-12 silicate will be partly dissociated, and above pH 12 dissociation will be complete. The dissociated and non-dissociated form can be separated by paper chromatography as described in the experimental section. Non-dissociated silicate will move faster during elution than the dissociated form, resulting in a spot for the non-dissociated form with high R_F and one for the dissociated form with low R_F. The pH determines the degree of dissociation of the silicate molecules, and hence the size of the two spots.

^{31}Si solution (pH 7-12) was added to chromatography paper, as described in the experimental section. After development two radioactive spots were found (spot 1, R_F 0.1 and spot 2, R_F 0.9) containing an amount of ^{31}Si dependant on the pH of the solution. An increase in pH results in a higher activity of ^{31}Si in spot 1 and a decrease of activity in spot 2. The fraction of dissociated and non-dissociated silicate is calculated and the results are summarized (Figure 6). These results are in accordance with the results of the gel formation experiment, and confirm the silicate chemical form of the ^{31}Si tracer.

It is possible that during neutron irradiation of the target material the formed ^{31}Si reacts with the phosphoric acid as a result of the heating up,[25] influencing the chemical properties of the ^{31}Si nuclide. To investigate whether this is feasible ^{32}P is brought into contact with 0, 1 or 10 mM silicate and incubated for 5 hours at room temperature (20 ^{o}C) or at 115 ^{o}C as described in the experimental section. The high temperature is chosen to cover for a possible rise of temperature in the reactor during irradiation, the incubation time is equal to the irradiation time in the tracer production. After incubation the solutions were treated with Method 1, and the fraction of ^{32}P that remained in solution was determined. The results showed no influence of silicate concentration or temperature on the fraction ^{32}P that remained in solution (Table 4). In all cases 1.4 to 1.5 % of ^{32}P was not precipitated, which equals the amount found in Method 1 as described above. So it is unlikely a phosphorus-silicon complex is formed during irradiation.

Experimental

Chemicals

The following chemicals were used: phosphoric acid (H_3PO_4, Fluka 76922), silver acetate (CH_3COOAg, Fluka 85140), barium carbonate ($BaCO_3$, Fluka 11729), lanthanum nitrate ($La(NO_3)_3$, Merck 5326), and sodium metasilicate (Na_2SiO_3, Aldrich 30,781-5). All chemicals were at least of analytical grade, and silicon was not on the list of impurities

Preparation of the radionuclide

The target material, 100 mg (1 mmol) orthophosphoric acid (H_3PO_4) was solid packed in small polyethylene tubes which were sealed at both ends. These tubes were placed in polyethylene “rabbits” and pneumatically transported to a position close to the core of the 2 MW swimming pool research reactor of the Reactor Institute Delft, Delft University of Technology, The Netherlands. The targets were neutron irradiated for 5.0 h. The neutron fluxes at the target position were about 5.1×10^{12} $cm^{-2}s^{-1}$ for thermal and 3.7×10^{11} $cm^{-2}s^{-1}$ for fast neutrons.

^{31}Si and ^{32}P activities were measured in 5 ml (diluted) sample using a LKB liquid scintillation counter (β^- radiation, 98% detection efficiency or Čerenkov light, 39% detection efficiency). For determination of β^- radiation 15 ml Ultima Gold LSC cocktail was added to the sample. Čerenkov light detection was used in cases when chemical conditions (like low pH) gave problems with the LSC cocktail. Moreover, ^{31}Si was also determined in a well-type Ge-Li-detector (γ radiation). The radioactive decay of the samples was followed over time in order to calculate the initial ^{31}Si and ^{32}P activities at the end of the target bombardment. Moreover, half-lives were checked to ascertain for the absence of contaminations by mathematical fitting the half-live times to the decay of the radionuclides over time using MicroMath Scientist software.

Removal of phosphate, including ^{32}P from the radionuclide solution

Phosphate (including ^{32}P) was removed from the target material solution by precipitation with barium carbonate (Method 1), silver acetate (Method 2) or lanthanum nitrate (Method 3), or by anion exchange separation with Dowex resin (Method 4) or prefabricated columns (Method 5).

Method 1. The irradiated H_3PO_4 was dissolved in 2.0 ml deionized water and transferred to a plastic centrifuge tube. To the solution 100 µl 1.0 mol/l HCl was added, followed by 0.35 g (1.7 mmol, 10% excess) solid $BaCO_3$ causing CO_2 release and a white precipitate, probably barium hydrogen phosphate ($BaHPO_4$, $K_s = 10^{-6.74}$).[26] After 5 min. CO_2 escape had stopped and the solution was filtered (membrane filter, pore size 0.45 µm). The pH was increased by the addition of 150 µl 1 mol/l NaOH to lower the K_s of barium phosphate in order to precipitate the remaining barium and phosphate ions, followed by filtration to remove the precipitate particles.

Method 2. The irradiated H_3PO_4 was dissolved in 2.0 ml deionized water and transferred to a plastic centrifuge tube. 0.60 g (3.3 mmol) solid silver acetate was added to the radioactive solution causing a yellow precipitate, probably silver orthophosphate (Ag_3PO_4, $K_s = 10^{-16.1}$).[1] After 5 min. the suspension was filtered (membrane filter, pore size 0.45 µm), and 0.03 g (0.5 mmol) NaCl (solid) was admixed to the filtrate in order to react with the remaining silver ions and form AgCl ($K_s = 10^{-9.75}$).[1] A final filtration removed the precipitated particles.

Method 3. The irradiated H_3PO_4 was dissolved in 3.0 ml deionized water and transferred to a plastic centrifuge tube. To the solution 200 µl 1 mol/l NaOH was added, followed by 0.40 g (1.2 mmol) solid lanthanum nitrate $La(NO_3)_3$ yielding a white precipitate (lanthanum orthophosphate $LaPO_4$, $K_s = 10^{-25.75}$).[24] The suspension was filtered (membrane filter, 0.45 µm) and 100 µl 1 mol/l NaOH was added to the filtrate to react with the remaining La ions and form $La(OH)_3$ ($K_s = 10^{-20.06}$).[27] A second filtration yielded a filtrate free of precipitate particles.

Method 4. The irradiated target material (100 mg) was dissolved in 2.0 ml water. Dowex 8x2 anion exchange resin was soaked in ultrapure water, washed with 8.0 mol/l HNO_3 and rinsed with water until neutral pH. A plastic column (Bio-Rad Econo-pac) was filled with 10 ml resin slurry. The resin was loaded with 10 ml

5.0 mol/l NaCl solution and rinsed with 10 ml 100 mmol/l TRIS in ultrapure water (pH 5.0). The solution of the irradiated target material was adjusted to pH 5.0, added to the top of the resin and eluted with 10 ml 100 mmol/l TRIS in ultrapure water of the same pH. The first 4 ml eluent was discarded, the following 6 ml yielded the purified product.

Method 5. The irradiated target material (100 mg) was dissolved in 2.0 ml water. Prefabricated anion resin columns (Accell™ Plus QMA) were purchased from Waters (Etten Leur, The Netherlands). Before use the columns were rinsed with 5 mol/l NaCl (4 ml/ml bed volume) and water of the desired pH (8 ml/ml bed volume). The solution of the irradiated target material was adjusted to the desired pH with 1 mol/l NaOH and added to a column (max. 1 ml solution/g resin). The eluent was kept for activity determination.

Analyses

Silicate concentrations were determined with inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer OES Optima 4300DV). The spectrometer was calibrated with Merck CertiPUR silicon standard solution (1703).

X-ray diffraction measurements were performed on a Bruker-AXS type D5005 diffractometer, equipped with a Huber CuKa1 incident-beam monochromator and Braun Position Sensitive Detector. The measurement range was 15-70 degrees 2-theta with a stepsize of 0.039 degrees and a counting time per step of 1 s. The specimen consisted of a thin layer of powder on a Si single crystal wafer with orientation <510> (a 'zero-background' substrate).

Gel formation

A 5.0 ml 1.5 mol/l Na_2SiO_3 solution was added to a tube together with 100 µl $^{31}Si/^{32}P$ solution (refined by Method 1). Gel formation was started by adding 10 ml 0 - 3.0 mol/l HCl solution to the tube. The final concentrations in the tube were 500 mmol/l Na_2SiO_3 and 0 – 2.0 mol/l HCl. It is crucial the HCl solution is slowly added against the wall of the tube without producing any turbulence or mixing in the liquid. Disturbing the liquid, e.g. by shaking, retards the start of the polymerization reaction for several hours. After HCl addition the tubes were left undisturbed for 15 minutes to allow the gel formation reaction to start. Then the tubes were shaken to yield a homogeneous mixture of gel and liquid and allowed to stand for another 15 minutes to complete the polymerization reaction. After centrifugation at 1500 g the supernatant was added to a counting vial for activity determination. The gel pellet was dispersed in 10 ml water and the mixture was added to a counting vial for radioactivity determination. The volume and density of gel and supernatant were determined by weighing.

Paper chromatography

Paper chromatography was carried out on tracer solution at pH 7-12. ^{31}Si solution (refined by Method 1) was added to chromatography paper (Whatman, Maidstone, England), allowed to dry, and eluted in 2-propanol (70%), water (10%), 20% trichloroacetic acid (20%) and 25% ammonia (0.3%) mobile phase as described by Hettler[28] until the front had reached about 75 % of the paper. After drying the paper was put on a linear transport system and the elution profile of the tracer (^{31}Si and ^{32}P) was determined using a GM detector. After complete decay of ^{31}Si (after at least two days) ^{32}P activity was determined in the elution profile to calculate the original ^{31}Si activity in the separate spots.

Interaction of ^{32}P with non-radioactive Si

^{32}P solution was prepared by irradiating 100 mg H_3PO_4 as described above followed by dissolving the target material in 2.0 ml deionized water. The solution was kept for at least 2 days allowing ^{31}Si to decay completely. Subsequently 2.9 ml solution containing 50 mg/ml H_3PO_4 and 0, 1.0 or 10 mmol/l Na_2SiO_3 was added to a counting vial together with 100 µl ^{32}P solution. Plastic counting vials were used because of the good heat and pressure resistance. The vials were closed and incubated at 20 or 115 ^{o}C for 5 hours. After incubation the liquid was added to plastic centrifuge tubes and possible loss of water by evaporation was compensated for. 1.0 ml sample was used for radioactivity determination, and precipitation with barium carbonate was carried out on the remaining 2.0 ml liquid. Separation of precipitate and solution was performed by centrifugation at 1500 g. The supernatant and pellet were added to counting vials for radioactivity determination. The volume of the supernatant was determined by weighing.

Conclusion

All refining methods are suitable for tracer production, except Method 3 (lanthanum nitrate precipitation), which resulted in 99.5 % loss of ^{31}Si, probably by co-precipitation. The results show the specific activity is determined by the presence of silicon as a contaminant in chemicals and materials. Methods 4 and 5 (anion exchange) have the advantage of the highest removal of ^{32}P, but Method 4 is more time consuming than the precipitation methods. Probably this drawback can be overcome by applying a vacuum to increase the elution speed. Method 5 is a fast method with the highest recovery of ^{31}Si and removal of ^{32}P. However its specific activity is lowest of all methods, and the costs are relatively high compared to the other methods. If chemical precipitation is used Method 1 (barium carbonate precipitation) is preferred over Method 2 (silver acetate precipitation) because barium is less toxic than silver. Moreover acetate, the counter ion of silver stays in solution and can be consumed by many organisms as a substrate and interfere with the experiments, whereas carbonate, the counter ion of barium escapes as CO_2 during precipitation. The presence of a minor amount of ^{32}P in the tracer should not be a problem as long as it does not interfere with the experiments (especially when phosphate concentrations in the media are relatively high). However it is mandatory to do more activity determinations on one sample to correct for the contribution of ^{32}P to the signal.

The experiments show ^{31}Si has the silicate chemical form, which means the tracer is chemically identical to natural silicon and can be used for studies of biological systems. In conclusion, the purification Methods 1, 4 and 5 yield ^{31}Si tracer of high activity and high specific activity that are usable in biosystem studies. The choice depends on the needs of the experiment. When a high activity per volume is needed Method 1 is preferred, whereas Method 4 yields the highest specific activity, but is more time consuming.

Acknowledgements

The authors want to thank Niek van der Pers of Materials Science and Engineering of the Delft University of Technology for the X-Ray diffraction analysis and Jorrit Heikamp for experimental help.

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Tables and Figures

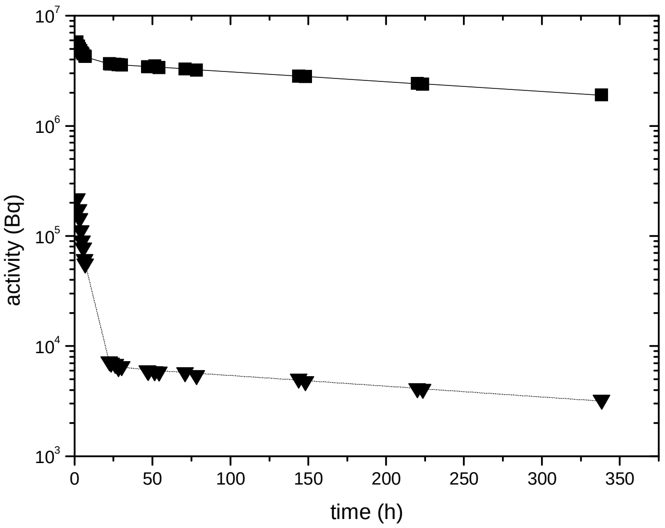

Figure 1. The ^{31}Si and ^{32}P activity in irradiated target material over time (■ not purified, ▼ purified by Method 1, lines: half life time fit of data)

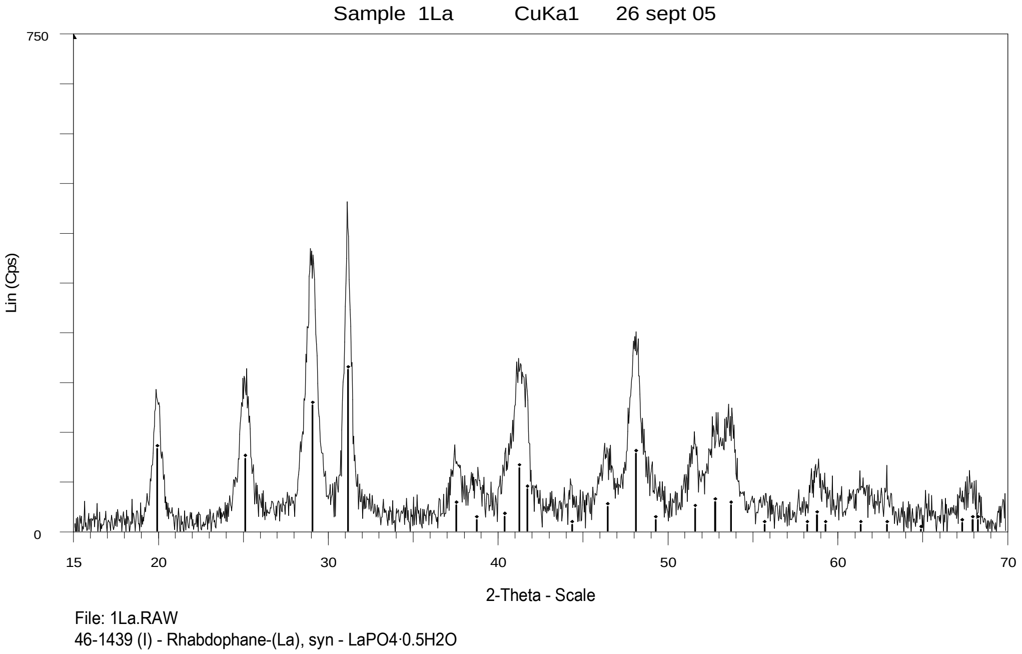

Figure 2 :Typical X-ray diffraction spectrum of $LaPO_4$.

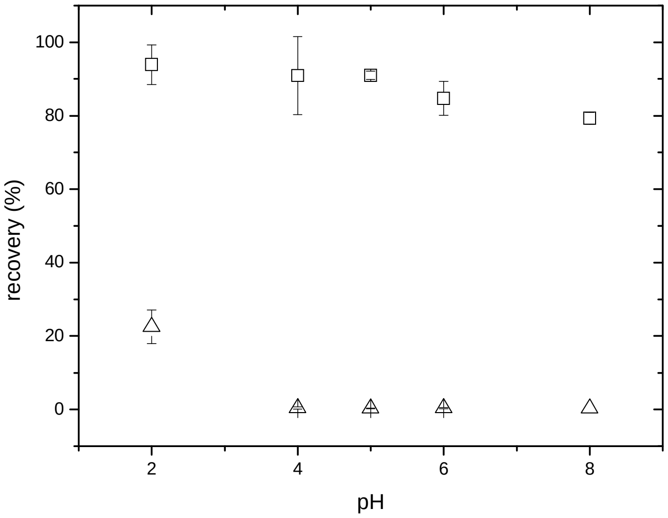

Figure 3 : Recovery of ^{31}Si (□) and ^{32}P (Δ) from the target solution as a function of pH after treatment with anion exchange (Method 5) (n=3, ± sd).

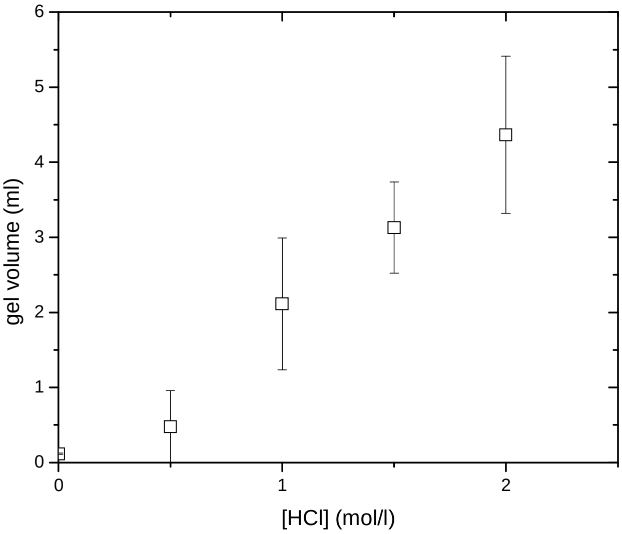

Figure 4: Gel volume obtained in 0.5 mol/l Na_2SiO_3 as a function of HCl concentration (n=4, ± sd).

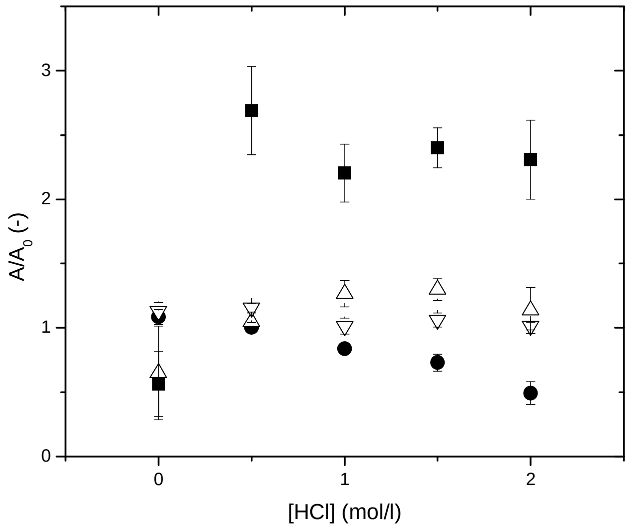

Figure 5: Activity (A) per ml gel or per ml supernatant after gel formation as compared to the activity (A_0) per ml at the start (■ ^{31}Si gel, ● ^{31}Si supernatant, Δ ^{32}P gel, ∇ ^{32}P supernatant, n=4, ± sd).

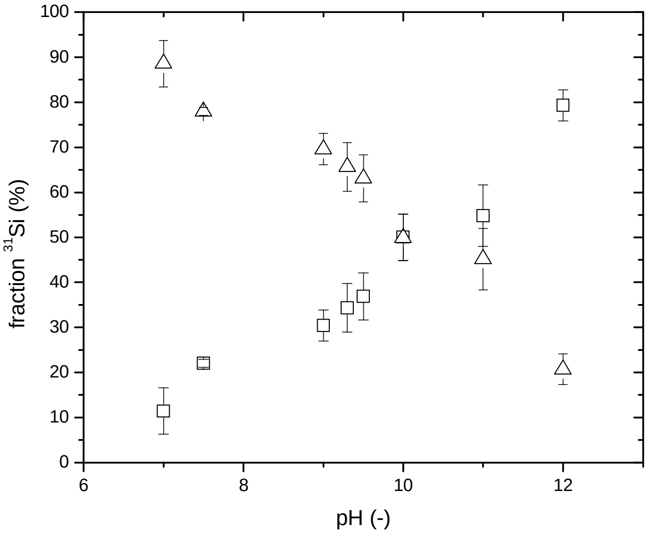

Figure 6: Activity of ^{31}Si in elution profile as fraction of the total activity of ^{31}Si as a function of pH, determined using paper chromatography (□ RF 0.1 dissociated form, Δ RF 0.9 non-dissociated form, n=3, ± sd).

Table 1: Preparation of the radionuclides ^{31}Si, ^{32}Si and ^{32}P and their physical data,[8,9,10] *recalculated value[10].

nuclide	**^{31}Si**	**^{32}Si**	**^{32}P**
half-live time	2.62 h	160 y	14.62 d
radiation type, energies	β^- 0.225 MeV (0.07%) β^- 1.49 MeV (99.9%) γ 1.27 MeV (0.07%).	β^- 0.213 MeV (100%)	β^- 1.710 MeV (100 %)
reaction, cross section	^{30}Si(n,γ)^{31}Si (0.108 barn) ^{31}P(n,p)^{31}Si (0.0312 barn)	^{37}Cl(p,2pα)^{32}Si (0.42 barn) ^{31}P(n,γ)^{32}P(n,p)^{32}Si (0.16 resp. 0.12* barn)	^{31}P(n,γ)^{32}P (0.16 barn)

Table 2: Properties of ^{31}Si tracer obtained by different purification methods. The remaining activity after purification is given as fraction of the original produced nuclide.

method	**nuclide**	**activity concentration Bq/ml**	**remaining activity at t = 0 %**	**[Si] mg/l**	**specific activity TBq/g**
1. precipitation ($BaCO_3$)	Si-31 P-32	$3.2 \pm 0.8 \cdot 10^{6}$ $2.8 \pm 1.4 \cdot 10^{4}$	74 ± 13 1.1 ± 0.2	0.67 ± 0.10	4.8 ± 1.4
2. precipitation (Ag-acetate)	Si-31 P-32	$1.8 \pm 0.05 \cdot 10^{6}$ $7.1 \pm 0.8 \cdot 10^{3}$	66 ± 22 0.25 ± 0.09	0.59 ± 0.06	3.0 ± 0.08
3. precipitation ($La(NO_3)_3$)	Si-31 P-32	$5.4 \pm 2.9 \cdot 10^{3}$ $6.7 \pm 2.5 \cdot 10^{2}$	0.5 ± 0.3 0.04 ± 0.01	$< 10^{-3}$	> 5
4. anion exchange (Dowex resin)	Si-31 P-32	$3.8 \pm 0.1 \cdot 10^{6}$ $3.2 \pm 0.3 \cdot 10^{3}$	43.9 ± 1.1 0.03 ± 0.003	0.21 ± 0.01	17.8 ± 0.4
5. anion exchange (Accell™ Plus Q)	Si-31 P-32	$1.1 \pm 0.1 \cdot 10^{6}$ $6.1 \pm 1.9 \cdot 10^{3}$	91.0 ± 1.1 0.4 ± 0.1	5.14 ± 0.09	0.21 ± 0.02

Table 3: Precipitating salts in refining methods 1 - 3 as determined by X-ray diffraction

method	**after addition of**	**formed precipitates**
Method 1	$BaCO_3$ + HCl NaOH	$BaHPO_4$ and $BaCO_3$ $BaCO_3$
Method 2	Ag-acetate NaCl	Ag_3PO_4 and Ag-acetate AgCl
Method 3	$La(NO_3)_3$ + NaOH NaOH	$LaPO_4$ no precipitate

Table 4: influence of temperature and silicate concentration on the fraction of not precipitated ^{32}P

[H_3PO_4]	**[Na_2SiO_3]**	**Fraction ^{32}P in supernatant**		**T-test**
		115 °C	**20 °C**	
g/ml	**mmol/l**	**%**	**%**	
0.05	0	1.50 ± 0.08	1.44 ± 0.05	ns
0.05	1	1.45 ± 0.10	1.41 ± 0.07	ns
0.05	10	1.48 ± 0.13	1.38 ± 0.01	ns

ns: not significant ($P>0.5$)