# The Separation of $^{32}$Silicon from Contaminating $^{3}$H and $^{60}$Co by Incorporation into Diatoms

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$^{32}$Si ($\beta^-$, 0,1 MeV, half life about 280 years) has been used, as for as we are aware, for the first time in biological and biochemical experiments. $^{32}$Si was incorporated by the pathway of the silicon metabolism into shells of two diatom species (*Cyclotella cryptica* and *Nitzschia* spec.) and reisolated by dissolving the shells. Contaminating isotopes $^{3}$H and $^{60}$Co with 10000 times more activity were largely removed by this procedure.

Silicon, the second most abundant element in the earth's crust after oxygen, is today regarded as an essential element in some unicellular organisms [1], in higher plants [2] and in animals [3]. Biochemical investigations of silicon metabolism using radioactive tracers have until now been restricted to $^{71}$Ge [4], $^{68}$Ge [5] and $^{31}$Si [6]. From the eight known nuclides of silicon (Fig. 1), only $^{32}$Si has a half life appropriate for longer lasting physiological and biochemical experiments. However this isotope has not been commercially available, for the radiochemical preparation of significant amounts is not simple. The present investigation was conducted with $^{32}$Si samples, produced for our laboratory in a reactor, with heavy contamination of about 80 mCi $^{3}$H and 7 $\mu$Ci $^{60}$Co *versus* 8 $\mu$Ci $^{32}$Si. $^{32}$Si decays to the daughter nuclide of $^{32}$P. After 14 days the activity of $^{32}$P reaches 50% of the activity of $^{32}$Si and after 140 days the same activity as $^{32}$Si.

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|---|---|---|---|
| Si 25<br>218 ms | Si 26<br>2.1 s<br>$\beta^+$ 3.8<br>$\gamma$ 0.82 | Si 27<br>4.2 s<br>$\beta^+$ 3.8<br>$\gamma$ | Si 28<br>92.21% |
| Si 29<br>4.70% | Si 30<br>3.09% | Si 31<br>2.62 h<br>$\beta^-$ 1.5<br>$\gamma$ | Si 32<br>280 a<br>$\beta^-$ ~0.1<br>no $\gamma$ |

Fig. 1. Isotopes of silicon [8]. For the stable isotopes $^{28}$Si, $^{29}$Si and $^{30}$Si the percentage of the presence are given.

Logarithmically growing cultures [7] of the centric diatom *Cyclotella cryptica* ($1.1 \times 10^7$ cells/ml) and of the pennate diatom *Nitzschia* spec. ($3.6 \times 10^6$ cells/ml) where used. The cells were transfered to a medium with a low $Si(OH)_4$-concentration, and 5 ml of this suspension was mixed with 5 ml of the neutral solution of the $^{32}$Si preparation from the reactor, containing 0.375 mg $SiO_2$/ml. The cells were incubated for 24 h at 20 °C in the light (10 000 cd/S × m$^2$) and gassing with a mixture of 1.75% $CO_2$ in air. After incubation the cells were harvested by centrifugation and washed thoroughly four times with water, leaving not more than $2.5 \times 10^3$ cpm in an aliquot (0.1 ml) of the supernatant (10 ml) *versus* $3 \times 10^7$ cpm in the original medium. The cells were then ashed in a muffle furnace for 3 h at 800 °C. The soluble ash and the silica shells of the diatoms were separated by centrifugation and wash-

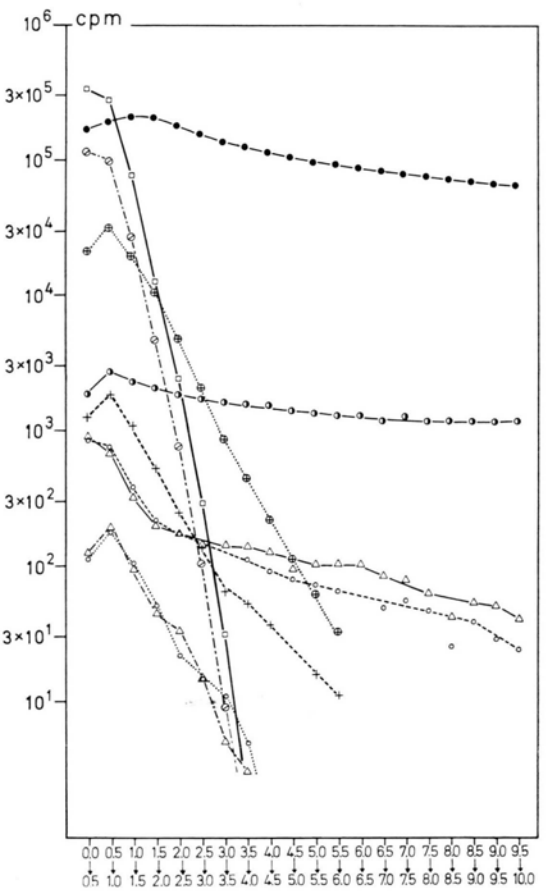


Fig. 2. Energy spectra, measured in a LSC-Packard 3380, with 5 ml dioxane-scintillator (4 g DPO, 74 mg POPOP and 100 g naphthalene/1 dioxane), Cerenkov-counting without scintillator. $^{32}$Si, $^{3}$H, $^{60}$Co-sample from the reactor (□), $^{3}$H-standard (ϕ), $^{60}$Co-standard (◑), $^{32}$P-standard (●), shell-preparation from *Cyclotella cryptica* (○), shell preparation from *Nitzschia* spec. (△), Cerenkov-counting $^{60}$Co-standard (+), Cerenkov-counting $^{32}$P-standard (⊕), Cerenkov-counting shell preparation from *Cyclotella cryptica* (○···), Cerenkov-counting shell preparation from *Nitzschia* spec. (△–·); abscissa: 0.0–0.5 etc. window units in the channels of LSC.

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ing nine times in water and once in 0.1 M NaCl, leaving not more than 50 cpm in an aliquot (0.1 ml) of the supernatant (10 ml). Finally the silica-shells of the diatoms were dissolved in 1 M NaOH at 100 °C. Radioactivity in samples of these two preparations were compared in a LSC with the original sample from the reactor and with pure standards of $^{3}H$, $^{60}Co$ and $^{32}P$ (Fig. 2).

The energy spectrum of the original sample is almost identical with the $^{3}H$ spectrum, as expected from the almost 10 000 times higher concentration of $^{3}H$ to $^{32}Si$. The ratio of counts at 0.5/1.0 window units to the counts at 4.0/4.5 units is about $4 \times 10^{5}$ : 1 with $^{3}H$, the ratio with both purified shell preparations is about 7 : 1, indicating a purification against tritium of more than a factor of $5 \times 10^{4}$.

The elimination of $^{60}Co$ by the biological method described should be rather efficient, for the incorporation of cobold into the shells of diatoms in significant amounts seems unlikely, though it was never determined exactly. The ratio of counts for the purified shell preparation at 0.0 – 0.5/9.5 – 10.0 window units is about 30 : 1 *versus* a ratio of less than 2 : 1 for a $^{60}Co$ standard, indicating a rather effective elimination of this nuclide. Fig. 2 also shows the significant differences between the energy spectra of $^{60}Co$ and $^{32}P$ standards, with scintillation counting and with Cerenkov counting of both isotopes.

The energy spectrum of the shell preparation with Cerenkov counting is very similar to that of the $^{32}P$ standard, for the energy of $^{32}Si$ is insufficient to give significant counts in Cerenkov counting.

The slight differences of shell preparations from the centric diatom *Cyclotella* and the pennate diatom *Nitzschia* between 3.5/4.0 and 9.5/10.0 window units are statistically significant. Further investigations of the silicon metabolism and of the shell composition of diatoms may help to explain also these differences.

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