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Enzyme Stabilization by Glass-Derived Silicates in Homeopathic Solutions

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LIGA, Redondo Beach, California, May 19, 2010

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Objectives

- Analyze the solutes leaching from glass in aqueous solutions
- Show that these solutes have enzyme stabilizing effects in very dilute and homeopathic solutions

Is there anything there?

- Until recently there has been very little investigation of possible solutes in high potency remedies
- High potency homeopathic remedies are thought to be solute free
- This is incorrect
- The chemical nature of compounds in mother tincture can affect the level of solute leaching from the glass
- Modern glass has aluminum oxides and boric oxides added to increase strength
  - High pH conditions will increase the amount of these compounds that dissolve from the glass

Measuring what is there

- Remedies made in water or water-ethanol mixtures in glass vials
- High potency remedies thought to contain nothing of starting material
- Persistent water structures have been proposed
- NMR have shown that homeopathic remedies are different from pure water but recent work has demonstrated that these finding are due to contaminants in the measuring cuvets
- Careful analysis with NMR has not shown any stable water structures in homeopathic remedies
- Commercial and hand-made remedies have been shown to contain micromolar concentrations of acetate, formate, lactate, acetone, ethanol and methanol
- Others have shown leaching of micromolar concentrations of minerals from the glass containers

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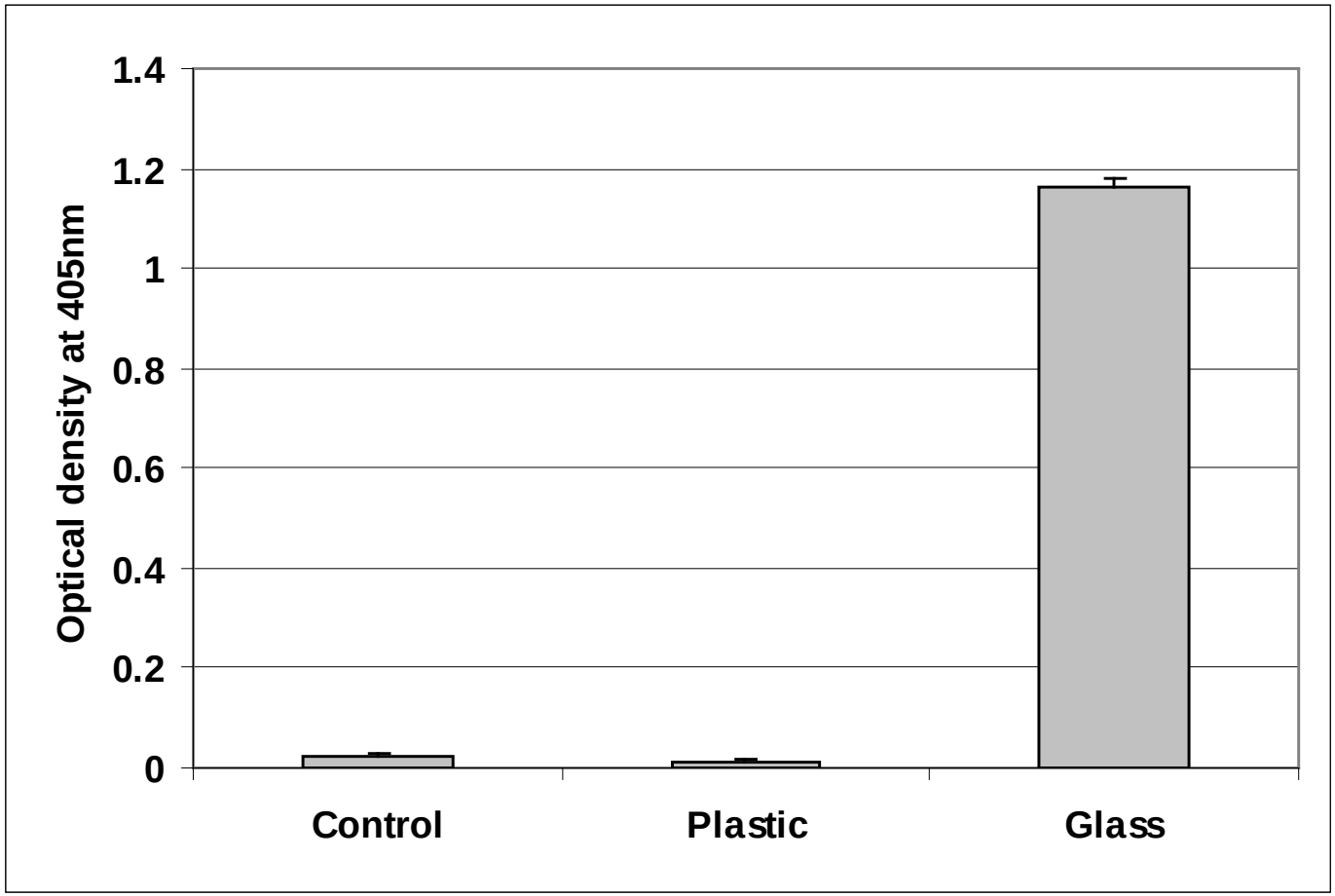
In our lab

- Developed enzyme-based bioassay of homeopathic remedies
- Found that enzyme activity preserved significantly longer in glass-exposed than plastic-exposed remedies
- Did not correlate in a consistent manner with the starting materials
- Increased succussion cycles did not significantly alter the effect.
- Determined the level of solutes present using highly sensitive analytical techniques including
  - elemental analysis
  - colorimetric silicate assays
  - enzyme assays
  - and scanning electron microscopy with elemental mapping in order to determine their solute content.
- Serially succussed and diluted (SSD) solutions made in glass vials contain micromolar levels of silicates, borate and sodium, and trace levels of other solutes
- Silicates are the most likely active agents in preserving enzymatic activity in dilute solutions

Methods

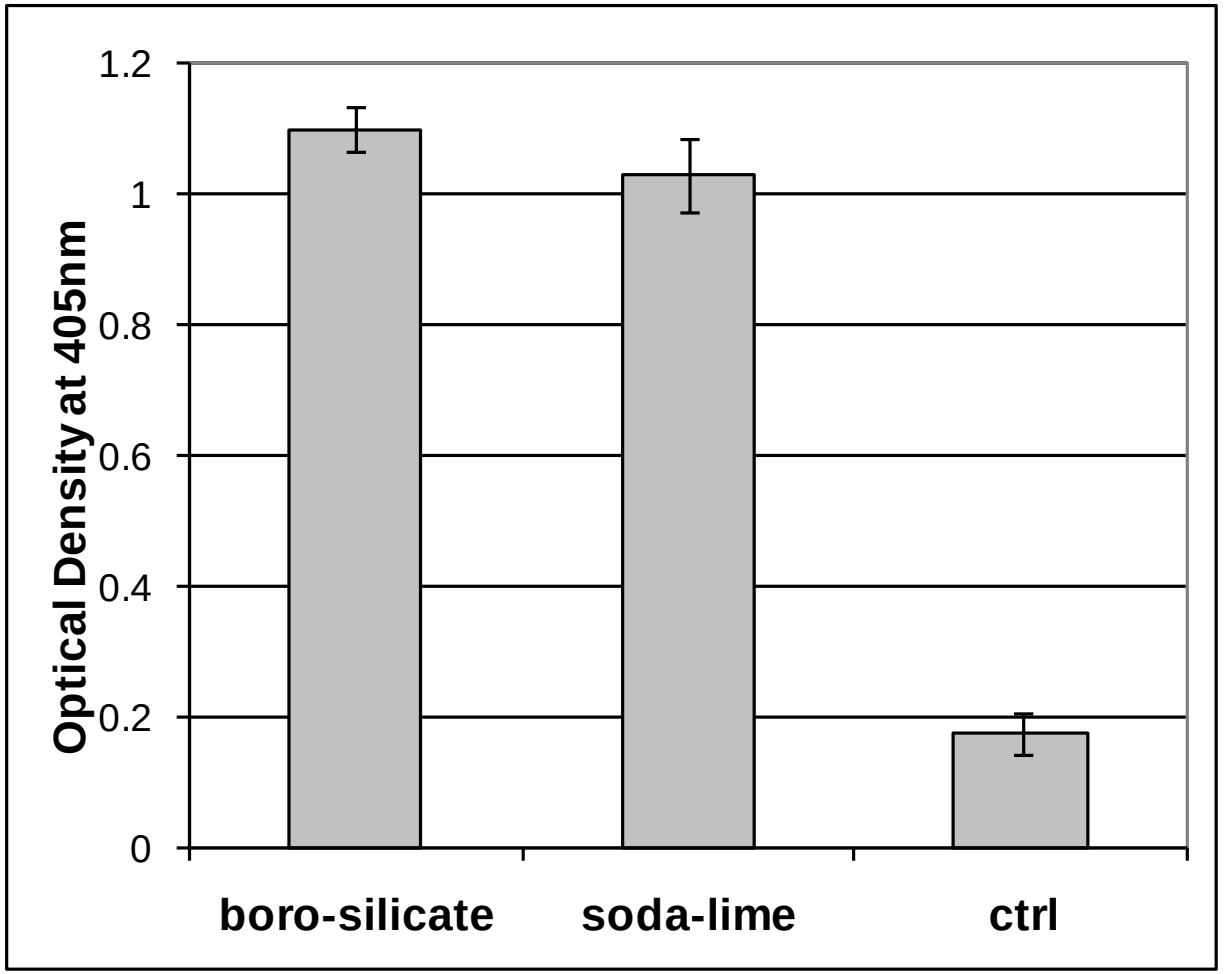
- SSD solutions of various solutes or water alone were prepared in plastic or glass
- Analyzed with acetylcholine esterase enzyme assays for ability to affect enzyme stability in dilute solutions
- Screw cap borosilicate glass tubes (24 ml) from VWR and soda-lime glass vials from Wheaton Scientific Products or 15 ml polypropylene centrifuge tubes
- Water glass (sodium silicate solution) containing ~27% SiO_2 and ~14% NaOH obtained from Sigma-Aldrich was used for making the SiO_2 solutions and the pH was adjusted to 7.4 with HCl.
- Trace elemental analysis to identify and quantify dissolved elements was performed by inductively coupled plasma-optical emission spectrometry (ICP-OES)
- Silicate concentrations in some solutions were estimated by the colorimetric molybdate method
- Scanning electron microscopy

Acetylcholine esterase stabilization



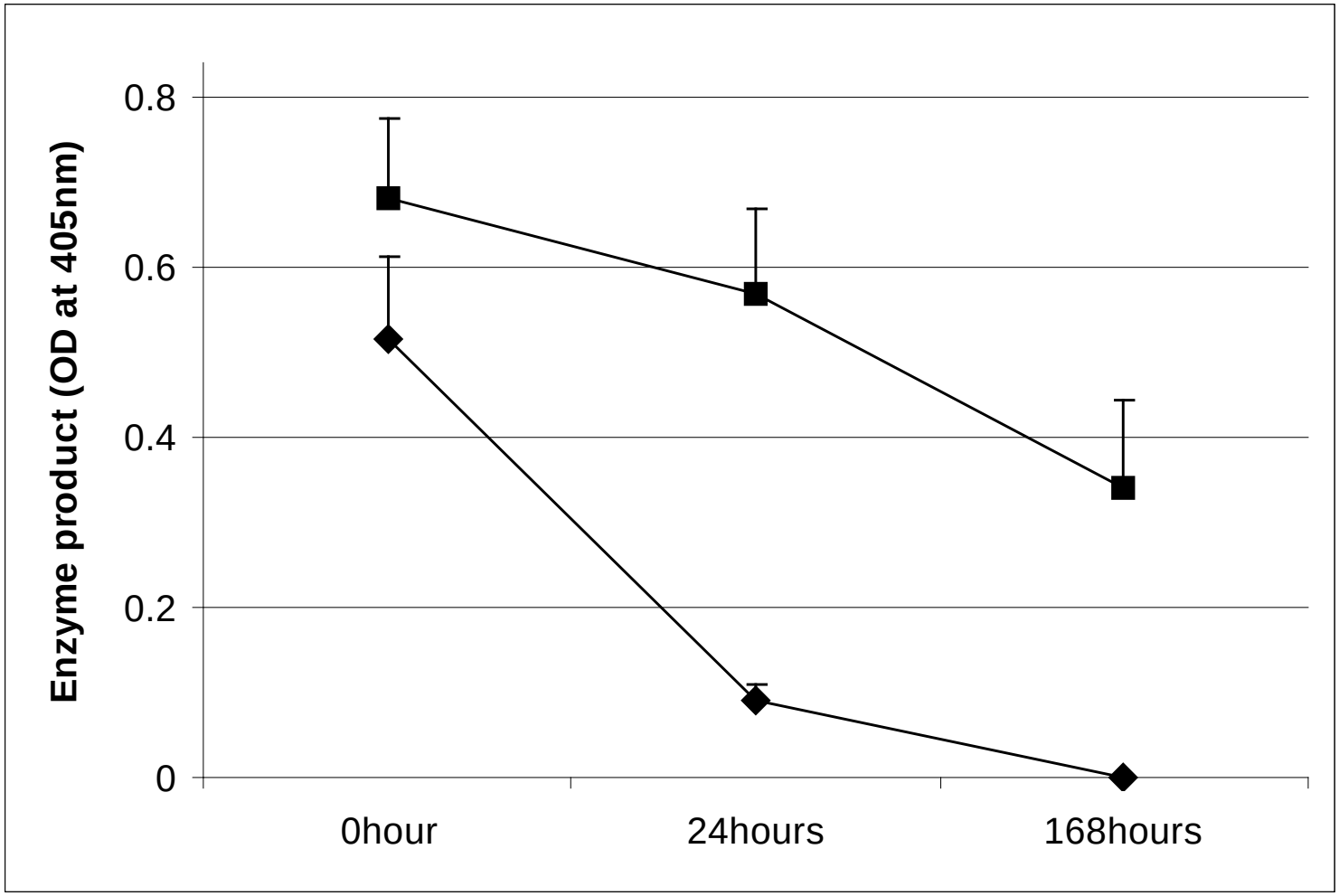
Dilute solutions prepared in plastic and glass compared with purified and deionized water (control). The solutions prepared in plastic (polypropylene) and glass (borosilicate) were succussed and diluted (120 succussions per dilution, thirty 100x dilutions, to yield 30c water preparations). Only glass-exposed solutions showed increased enzyme stabilization after 24 hours incubation at room temperature. Values are means ± SD (n=6, from 6 independently prepared samples).

Borosilicate versus soda-lime glass



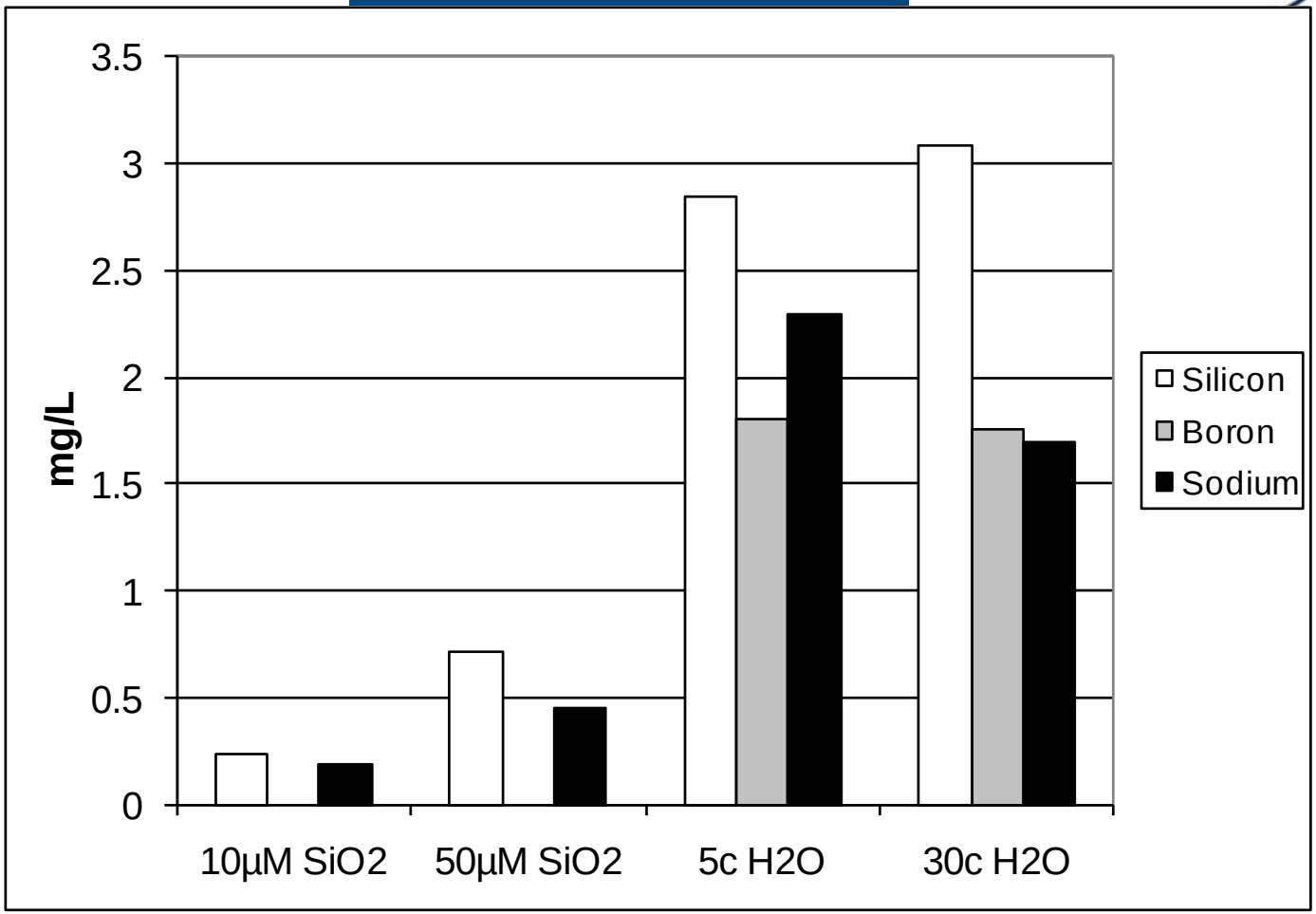
Stabilization of acetylcholine esterase 24 hr after addition to SSD preparations (15c glutamate) made in borosilicate versus soda-lime glass tubes, as compared with acetylcholine esterase dissolved in pure water kept in polypropylene tubes (ctrl). Both types of glass leached compounds into the water that acted to stabilize enzyme activity in solution relative to pure water. Values are means $\pm$ SD (n=6, from 6 independently prepared samples).

Acetylcholine esterase activity over time



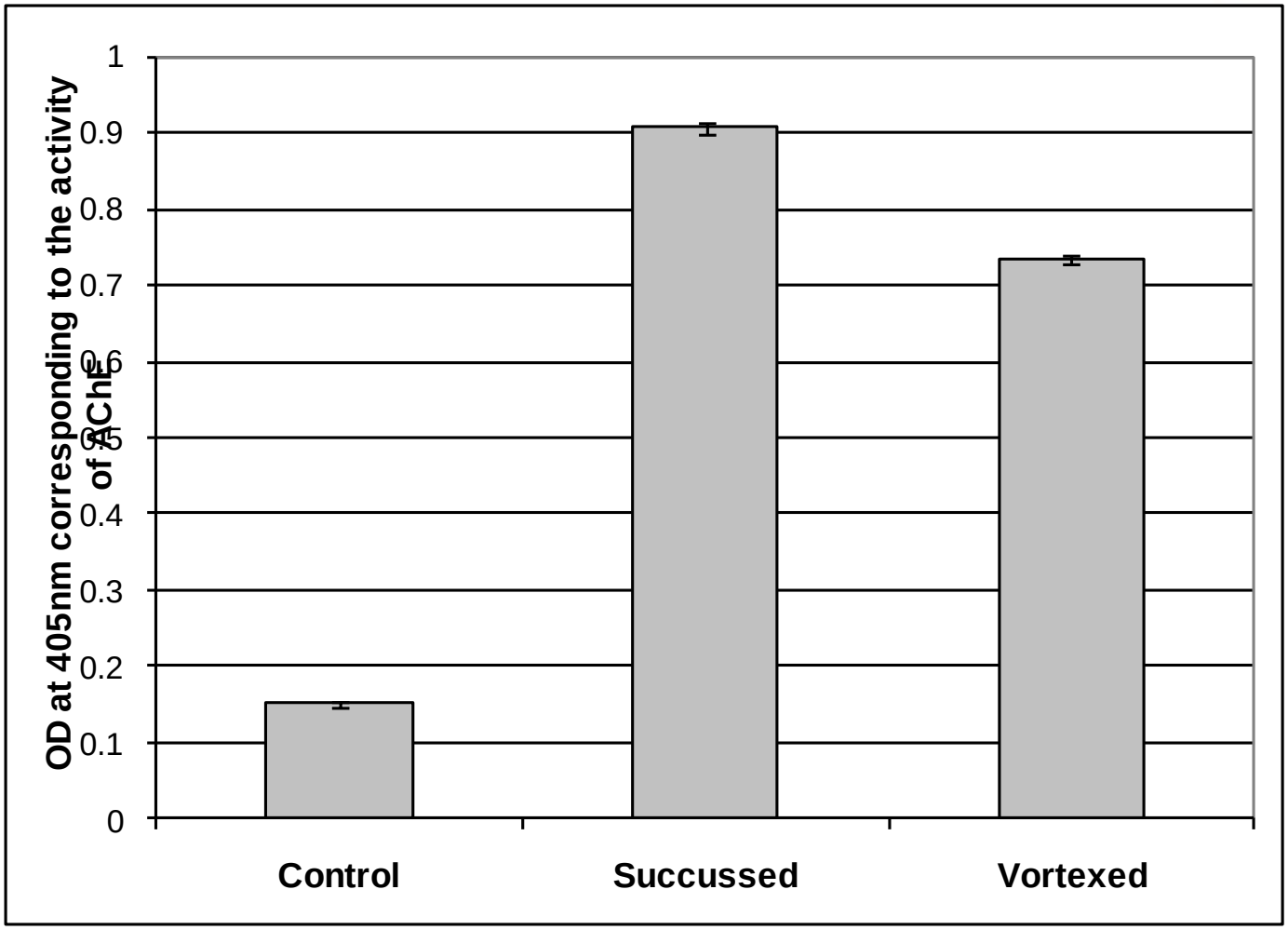
Acetylcholine esterase activity over time in pure water (not glass-exposed) and in SSD preparations (30c acetylcholine) made in borosilicate glass vials. Filled squares are acetylcholine esterase activity in SSD solutions made in borosilicate glass, filled diamonds depict activity in purified, deionized water. Values are means ± SD (n = 7 independently prepared samples, tested with 3 different stock enzyme preparations each, and each sample/enzyme prep mixture was assayed in triplicates at each time point).

Elemental analyses



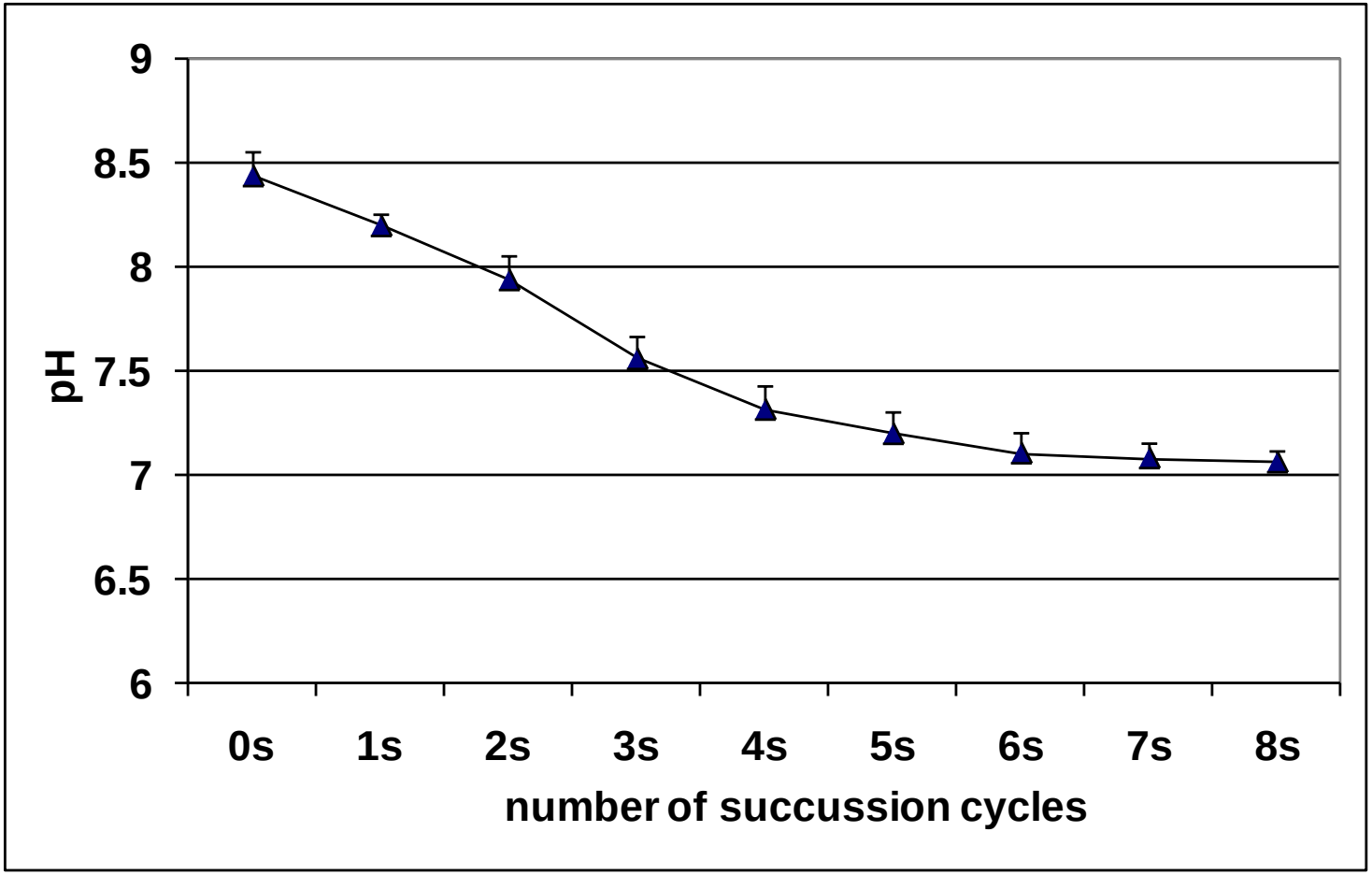
Elemental analyses (ICP-OES) of samples including water with soluble silicon dioxide added (10 and 50µM sodium silicate), and two SSD solutions made by shaking water 120 times each in 5 successive borosilicate glass tubes (5c H_2O) and in 30 successive borosilicate glass tubes (30c H_2O). The sodium silicate solutions contained sodium and silicon, but had undetectable levels of boron. In contrast, the SSD preparations made with deionized water in glass vials contained sodium, silicon and boron, and the silicon levels were approximately 4 times those found in the 50 µM sodium silicate solution. Values are from individual samples. Experiments were repeated 3 times with similar results.

Succussion vs. vortex mixing



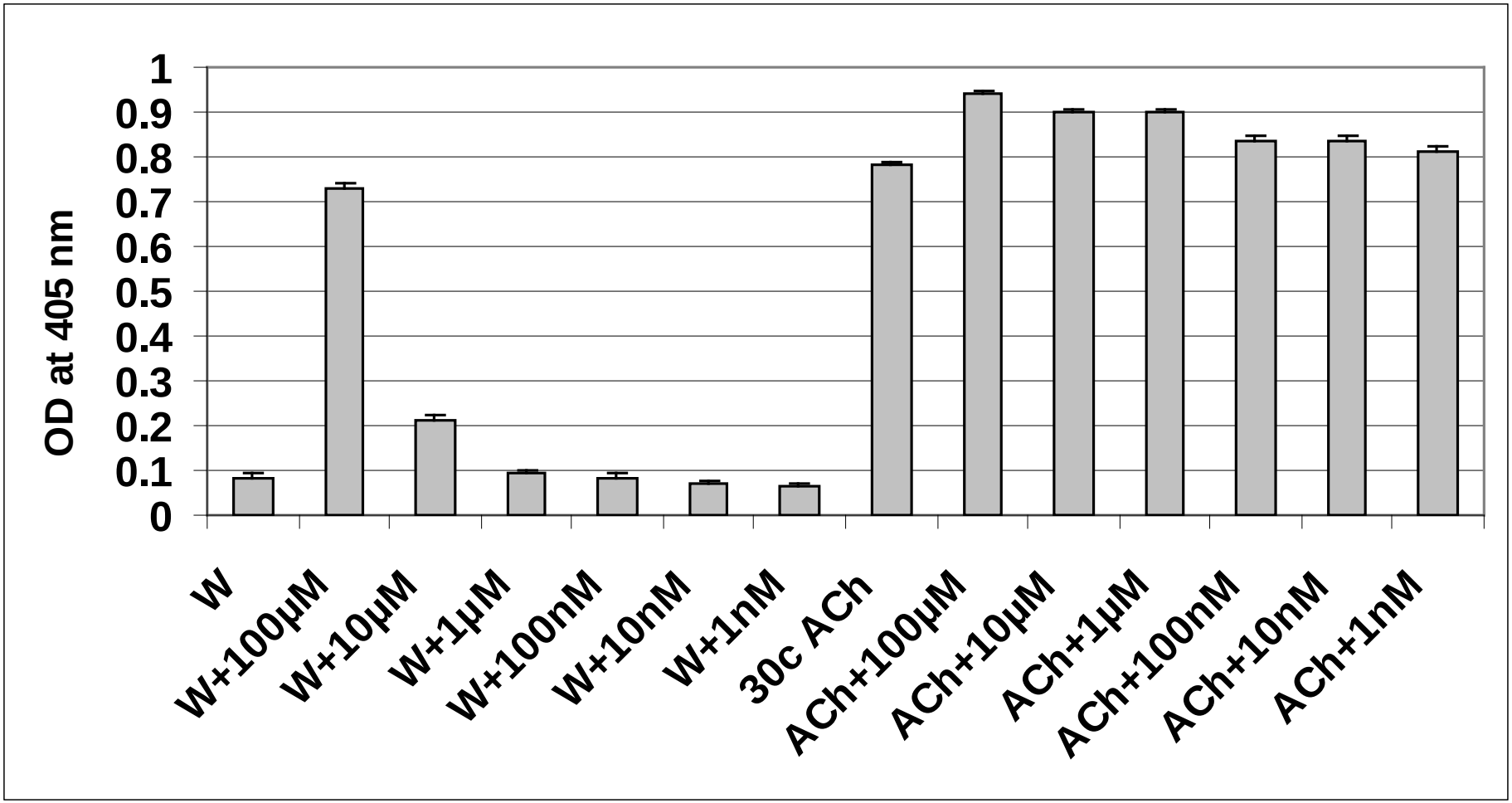
Acetylcholinesterase activity in pure water (control) and in water succussed in a borosilicate glass tube 120 times in 1 minute, compared with water that had been vortex mixed in the same type of tube for 1 minute. Enzyme activity was measured after 24 hours in solution at room temperature. Values are means $\pm$ SD (n=6, from 6 independently prepared samples). The vortex mixed samples were significantly different from the SSD samples as shown by 2-tailed t-test ($p < 0.001$).

Effect of succussion cycles on pH



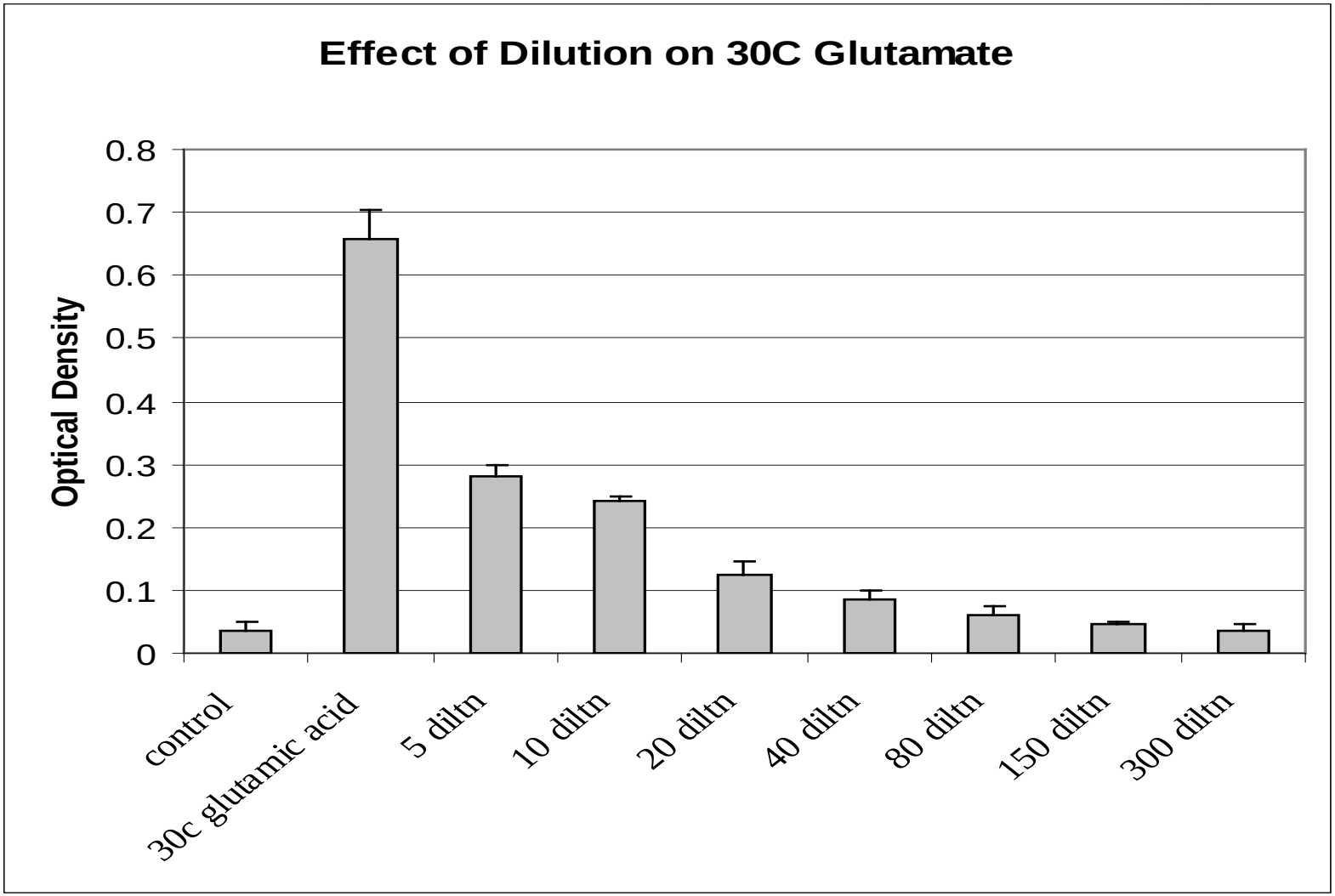
Effect of number of succussion cycles (s) on the pH of an aqueous solution made in a borosilicate glass tube. The same glass vials containing the same water were succussed and then tested for pH for 8 succussion cycles. The vials and the water in them were not changed between succussion cycles. Values are means ± SD (n=3, from 3 independently prepared samples).

Phosphate buffer compared to SSD



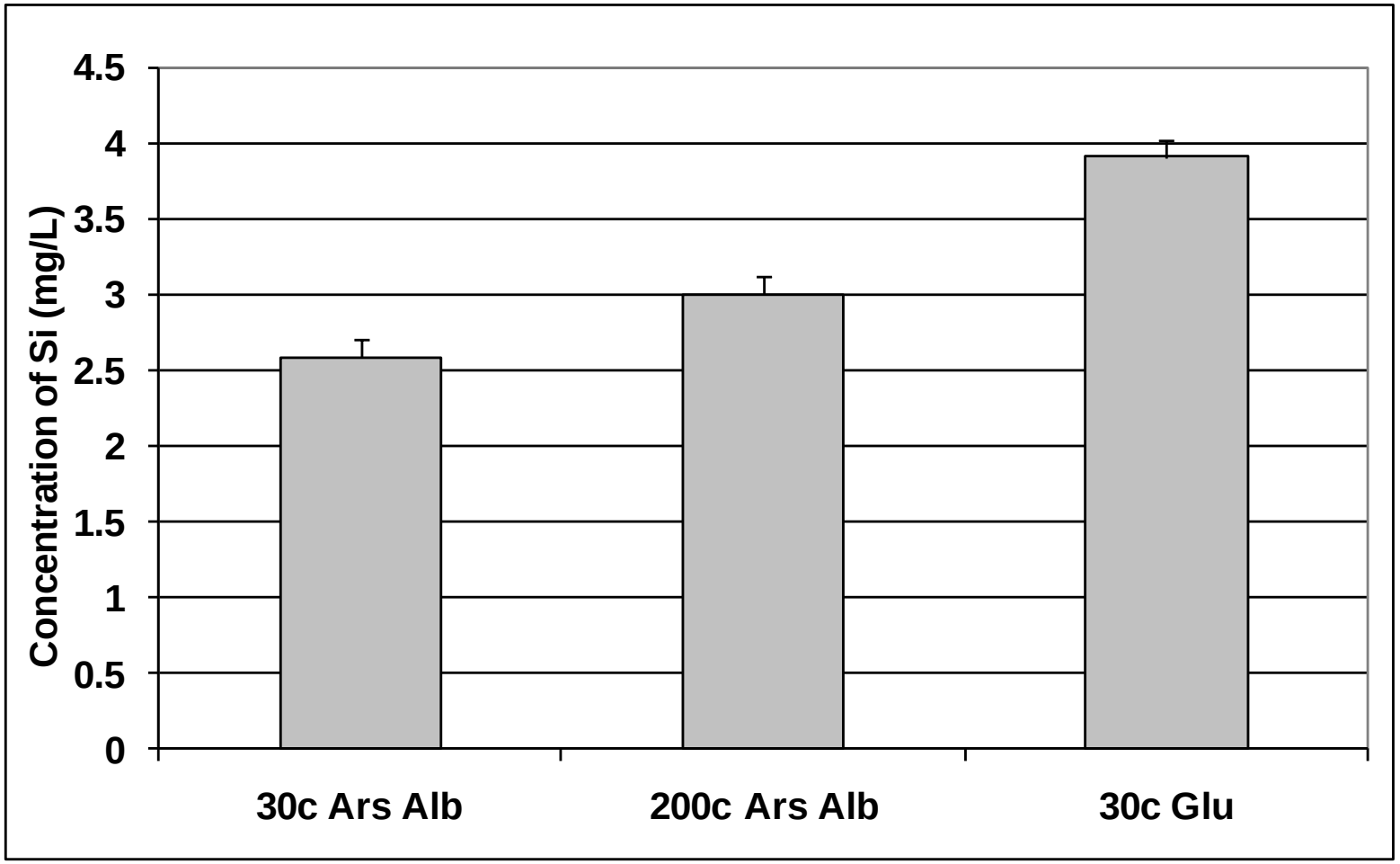
Phosphate buffer and SSD effects on enzyme stability after 24 hours. The enzyme stabilizing effect of an SSD preparation made with acetylthiocholine (30c ACh) was approximately the same as with 100 µM sodium phosphate buffer in water (W). Adding 100 µM sodium phosphate buffer to the 30c ACh solution (ACh+100 µM) increased enzyme stability more than 15% as compare with 30c ACh alone (n=6, ± SD, from 6 independently prepared samples).

Dilution tests



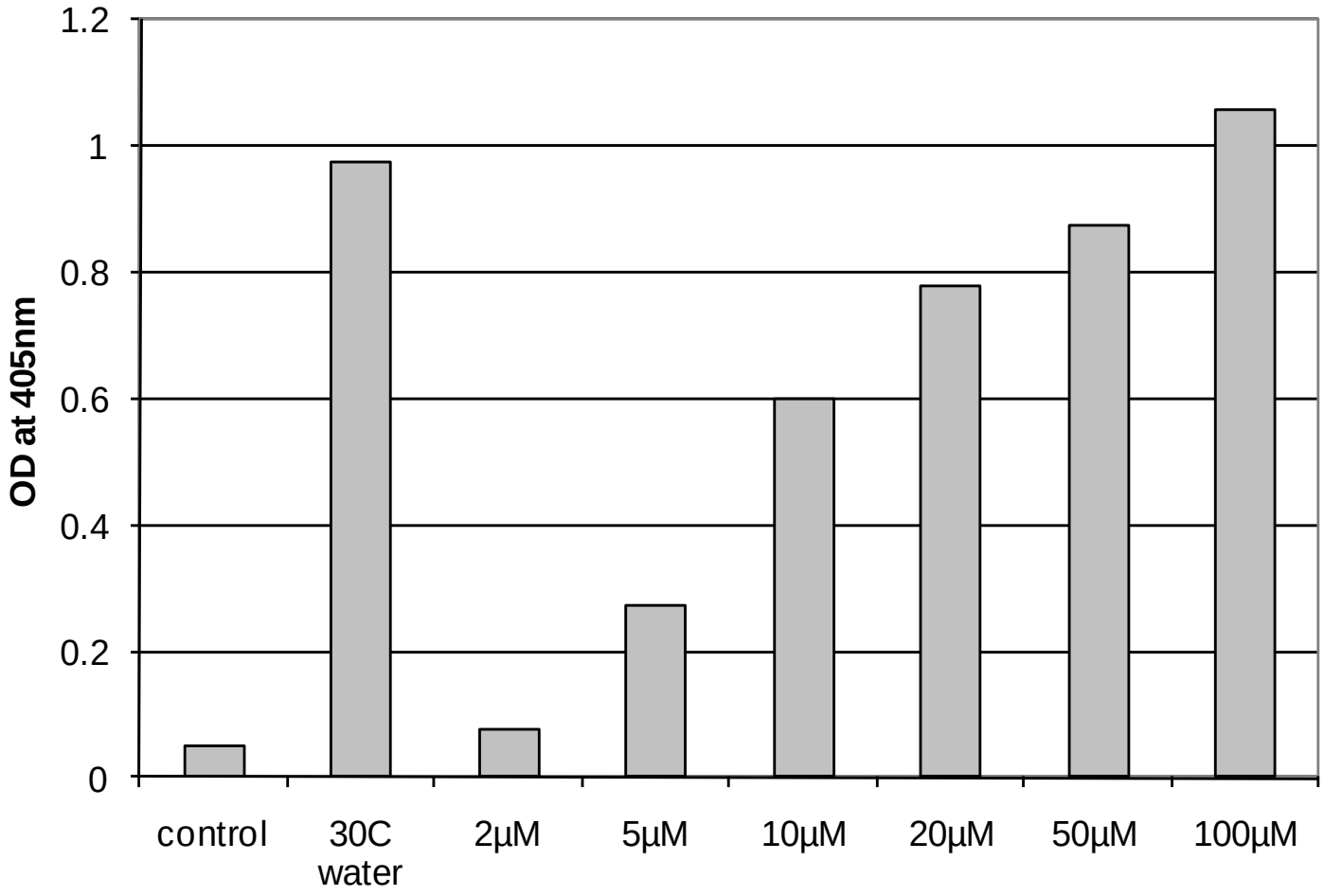
Acetylcholine esterase activity after 24 hour incubation in 30c glutamate stock solution, and a number of dilutions made and vortex mixed in plastic tubes. The 30c stock was prepared as described in borosilicate glass tubes, and then diluted (5 diltn = 5 fold dilution, etc.) with purified, deionized water (n=6, $\pm$ SD, from 6 independently prepared samples).

Silicate levels in homeopathic preparations



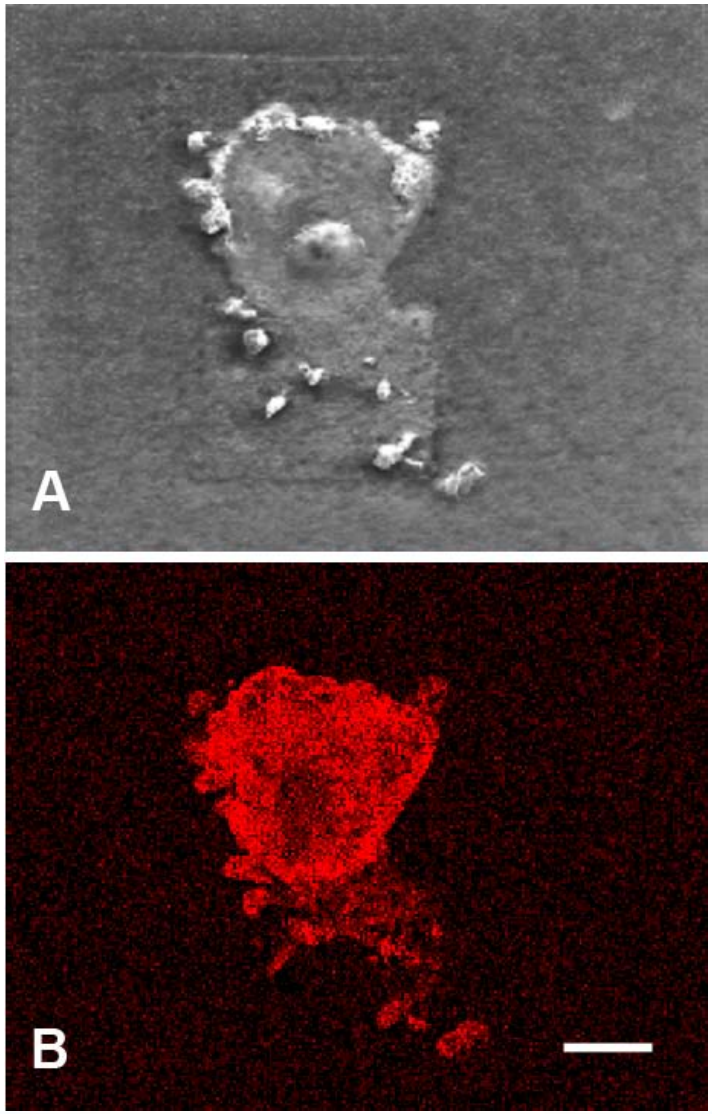
Molybdate assay for silicate content in 2 commercial homeopathic preparations (arsenicum album: Ars Alb) and 30c glutamate (30c Glu) prepared in our laboratory (n = 3 $\pm$ SD, from 3 independently prepared 30c Glu samples and 3 different commercial arsenicum album vials).

Sodium silicate vs. SSD preparations



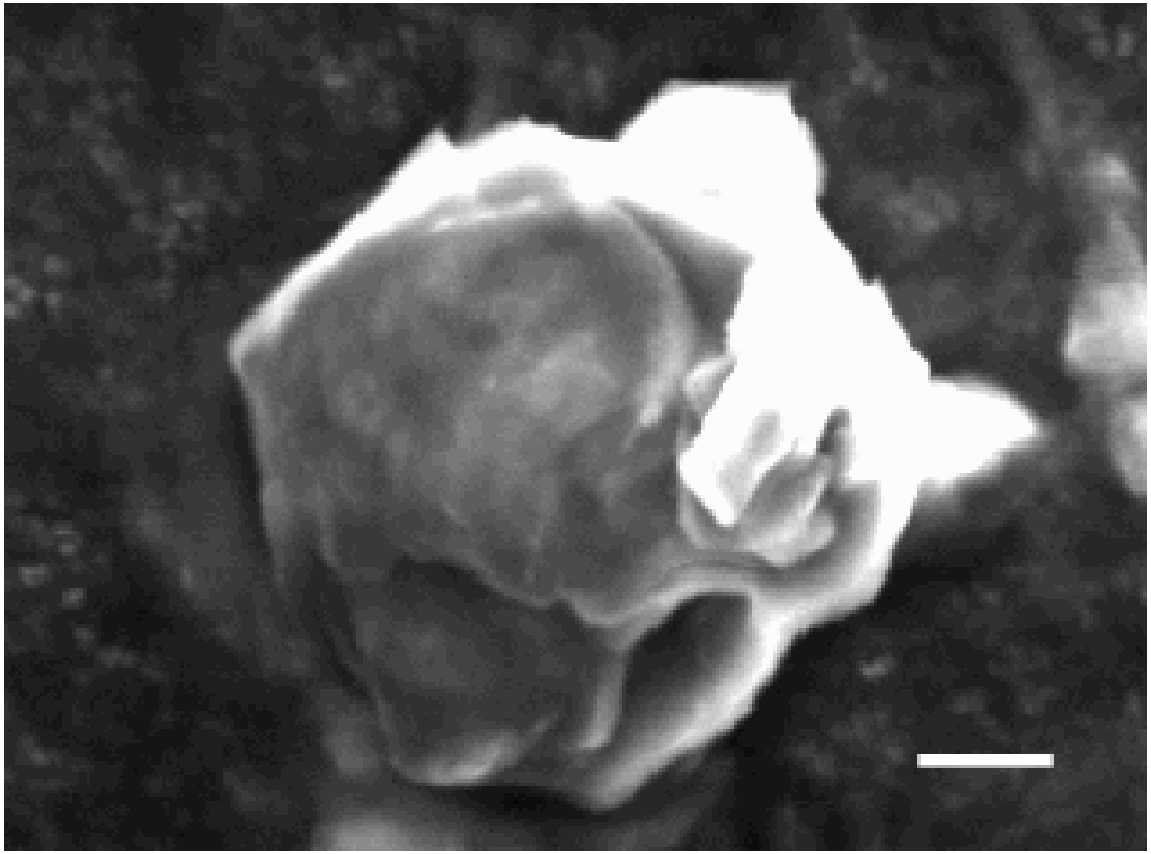
Effect of dissolved silicates (NaOH/silicate) on acetylcholine esterase enzyme activity in solution as compared with an SSD preparation made with purified, deionized water in borosilicate glass tubes (30c water). The silicate solutions (2 µM to 100 µM) were prepared in polypropylene tubes and were vortex mixed. A 100 µM sodium silicate solution had a similar enzyme stabilizing capacity as the SSD preparation made in borosilicate glass tubes (n=6 ± SD, from 6 independently prepared samples).

SEM and silicon mapping of lyophilized SSD samples prepared in glass vials



SEM image and silicon mapping of a patch of lyophilized residue from an SSD preparation. SEM image is shown in (A), and the corresponding silicon map is shown in (B). Colloidal silicate-containing particles can be seen around the edge of the dried residue. Bar = 50 µm.

Colloidal Silicate Particle



Scanning electron micrograph of a colloidal silicate particle lyophilized from a sample of an SSD preparation made in a borosilicate glass vial. Proteins bind tightly to colloidal silica particles in solution, and this has been reported to enhance enzymatic activity. Bar = 5 µm.

Results

- Water acts to dissolve constituents from glass vials
- Solutes derived from the glass have effects on enzymes in the resultant solutions
- Enzyme stability in purified and deionized water enhanced by serial dilution and succussion in glass containers but not plastic
- Mimic this effect in a dose-dependent manner with the addition of silicates to purified, deionized water used to dissolve enzymes
- Serially succussed and diluted solutions made in glass vials contain micromolar concentrations of boron, silicon, and sodium

Conclusions

- All glass exposed solutions, whether homeopathic or pharmaceutical in nature, contain micromolar concentrations of silicates and other solutes
- Silicates are known to be bioactive at millimolar concentrations
- Homeopathic solutions we measured do not have sufficient concentration of silicates to account for *in vivo* effects
- *In vitro* effects reported in literature may be influenced by the dissolved solutes we detected