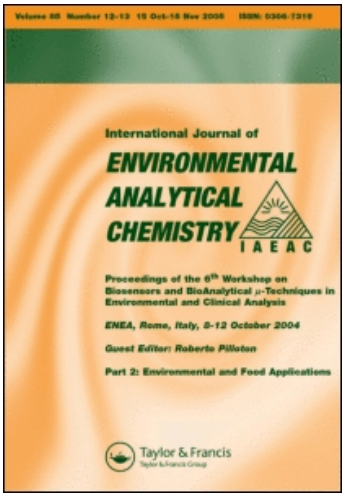

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A Rapid, Routine Atomic Absorption Spectrometry Method for the Determination of Selenium at Sub-Microgram Levels in Animal Tissue

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A Rapid, Routine Atomic Absorption Spectrometry Method for the Determination of Selenium at Sub-Microgram Levels in Animal Tissue

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KEY WORDS: Selenium, atomic absorption, animal tissues.

A method is reported for determining selenium (Se) in animal tissue through Schöniger combustion, Se reduction to H_2Se, and measurement by flame atomic absorption. The method provides several improvements over present hydrogen selenide generation apparatus and procedure. For the National Bureau of Standards Bovine Liver reference material containing 1.1 ± 0.1 ppm Se, recovery averaged 100% with a relative standard deviation of 15%. The minimum detecable quantity is 0.04 μg Se. Data from the literature and results of analyses in our laboratory indicate that the method is adequately sensitive for tissue samples of almost any origin. Overall, our method is sensitive, precise, accurate, and simple and fast enough to use economically in routine monitoring of wildlife samples.

The measurement of selenium (Se) in biological materials has become important for several reasons. Biochemically, Se is quite interesting, as no known enzyme requires Se yet it seems to replace sulfur in at least six mammalian enzymes.[1] Lately, Se has been hypothesized to act as an anti-oxidant, and its relationship with Vitamin E is of increasing interest.[2] Se is known to be a dietary requirement for poultry and many mammals (including man), with deficiency resulting in varied forms of necrotic tissue degeneration, yet Se is also extremely toxic to many forms of wildlife.[1-4]

Highly seleniferous soils occur in the western United States, and plants such as Se-obligate species of *Astragalus* and *Stanleya* may accumulate concentrations up to 1% dry weight.[2-4] Livestock that feed on these plants may exhibit such symptoms as congenital malformations, liver and kidney damage, and paralysis and weakness, a syndrome first described by Marco Polo in the 13th Century in China and commonly referred to today as "blind staggers" or "alkali disease".[1,2,4] Optimum dietary levels for mammals are in the range of 0.5–3.5 μg/g Se (as selenite) in feed,[2] with lower levels leading to deficiency and higher levels considered to be toxic.

Although concentrations of Se below 0.1 μg/g have only rarely been reported in tissues of fish, wild or domesticated mammals, or man,[1,5-10] Se is naturally present in vertebrates at varying and sometimes extremely low levels. Therefore an analytical method to determine Se in tissues should be able to measure submicrogram quantities and to discriminate differences at levels as low as 0.1 μg/g. A number of methods have been reported for the determination of Se in biological materials, using colorimetry,[11-13] fluorometry,[9,10,14,15] instrumental neutron activation analysis,[16,17] titrimetry,[3,18] or spark source mass spectrometry.[19] In determining low levels in tissue samples, a constant problem with these and other analytical methods is the difficulty of converting the material to a state suitable for measurement without substantial losses of volatile Se compounds. Those methods using Schöniger combustion [9,13-15] are reported to give nearly 100% recovery of added Se compounds. Unfortunately, due to consideration of cost, sample size requirement, sensitivity, complexity of equipment, or procedure, these Se referenced methods were judged unsuitable for use in routine monitoring of environmental samples. Instead, we developed a procedure combining Schöniger combustion with an atomic absorption (AA) spectrometry technique described originally by Holak[20] for determination of As as arsine and later by Dalton and Malanoski,[21] Fernandez and Manning,[22,23] and Hwang *et al.*[24] for determination of As, Sb, or Se as their gaseous hydrides. Our procedure is precise, accurate, sensitive, and simple, and fast enough to use economically in routine monitoring of wildlife samples.

MATERIALS

Apparatus

The Schöniger combustion apparatus is the same as that described by Okuno *et al.*[25] All measurements were made with a Jarrell–Ash Series 82–360 Atomic Absorption/Flame Spectrometer, equipped with a 10-mv full-scale Speedomax W Leeds and Northrup recorder.† The apparatus used

† Reference to trade names does not imply endorsement of commercial products by the Federal Government.

for H_2Se generation is shown in Figure 1. The burner was aligned to give maximum Se absorption (1.3 cm below the center of the light beam). The Se hollow-cathode lamp, Jarrell–Ash model 22938, was operated at a current of 15 ma on the pulsed high-intensity mode. Photomultiplier tube voltage was 780 kv. Other settings were: 1960.3 Å; slit width, 100 μ; He flow, 24 SCFH; H_2 flow, 3 SCFH.

Reagents

Analytical grade reagents were used. Standard Se solutions were made daily by diluting a 100 ppm stock solution (14.05 mg of SeO_2 dissolved in 100 ml distilled water). Other reagents were: stannous chloride solution (20% $SnCl_2 2H_2O$ w/v in conc. HCl), zinc granules (20 mesh), HCl solution (50% v/v), H_2SO_4 solution (25% v/v), and a H_2SO_4-HCl solution (12.5–25.0% v/v). Arthur H. Thomas black paper sample-wrappers (6471-Q25) were used for combustion.

PROCEDURE

Schöniger combustion

Combustion is performed by the method of Okuno *et al.*[25] Up to 1 g of wet tissue is dried over P_2O_5 and burned in a 2-L flask containing 10.0 ml of the H_2SO_4-HCl solution. After combustion and cooling, this solution is swirled to contact all of the interior surface of the flask and decanted into a 50-ml pear-shaped generation flask. The combustion flask and sample carrier are rinsed with enough H_2SO_4-HCl solution to bring the collected volume in the generation flask to 20 ml. Small bits of ash are allowed to enter the generation flask.

Standards for comparison are prepared by adding 1.0 ml of the appropriate standard solution to the generation flask followed by 9.0 ml of 25% H_2SO_4 and 10.0 ml of 50% HCl.

AA determination

To a standard or sample in the generation flask, add 0.5 ml of $SnCl_2$ solution and swirl to mix. Join the generation flask to the gas inlet of the vacuum-type distilling tube joint and fix in place by spring supports (Figure 1). Add about 2 g of zinc granules to the latex pipette attached to the male joint stem, join the male joint stem to the second neck of the reaction flask, and secure by a rubber band. In attaching the zinc-filled bulb, fold it over and hold it to prevent the zinc from entering the generation flask. Open the stopcock and

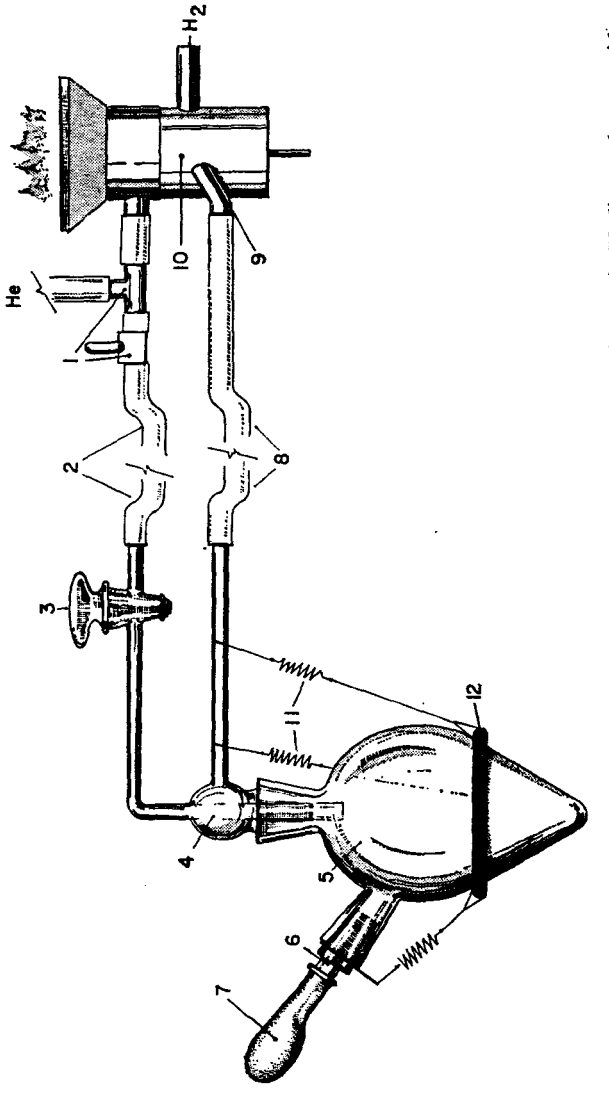

FIGURE 1 Apparatus—generation flask, gas flow system, and burner: (1) Tee in He line to burner with snap valve; (2) Imperial Eastman Poly-Flo tubing 44-P-1/4"; (3) two-way stopcock; (4) distilling tube joint T/S 14/20, gas inlet vacuum type; (5) generation flask, 50 ml 2 neck 14/20 T/S; (6) male ground glass joint 14/20 T/S; (7) laytex rubber pipette bulb; (8) Tygon tubing, formulation R3603, ID 1/4"; (9) drain from burner; (10) Jarrell-Ash Tri-Flame Burner JA82-368; (11) spring supports; (12) ring support for flask.

thoroughly flush the system with He, then close the stopcock and release the bulb so that the zinc enters the flask. Allow generation to continue until the optimum response is expected (as determined by previous experimentation); then open the stopcock to sweep the generated gases into the AA flame. The recorded response to the H_2Se is a sharp peak. Percent absorption is converted to absorbance and a standard calibration curve of absorbance vs. μg Se is prepared. The standard curve is corrected for the gas generation blank, and sample values are corrected for the blank values contributed from combustion.

RESULTS AND DISCUSSION

The generation apparatus described here is a considerable simplification over previous systems.[22-24] Adding a carrier gas bypass of Tygon tubing and eliminating the expansion balloon eliminate the need for a series of expensive three-way glass stopcocks, control valves, metering system, and elaborate glass tubing construction.

The use of He as a carrier gas, in place of the argon previously used,[22-24] significantly reduces flame noise and increases sensitivity about two-fold (Figure 2). However, to obtain a stable flame, gas flow must be approximately tripled with He. Optimum gas flow rates were found to be 24 SCFH for He and 3 SCFH for H_2.

The sensitivity and reproducibility of the AA instrument response were found to be highly dependent on the time period between adding the zinc and sweeping the generated gases into the flame. Hwang *et al.*[24] have discussed this phenomenon, postulating that decreases in sensitivity and precision are caused by incomplete reaction when the system is flushed before the optimum time and by dissolution of hydrides in the generation liquid and diffusion when it is flushed after the optimum time. Figure 3 compares responses from bleed with responses from sweeping; the curves were obtained by running replicate 1.0-μg standard solutions and sweeping at different times after the addition of zinc. Some bleed occurred when generated H_2Se was pushed into the flame by the sheer volume of hydrogen formed. Using a down-line reservoir of about 100 ml eliminated bleed but resulted in lower sensitivity on sweeping, perhaps due to dispersion of H_2Se within the carrier gas. The middle of the plateau region of the sweep curve corresponded with the peak of the bleed curve, and this point was chosen as the optimum generation time (OGT), since it gave the best compromise between sensitivity and reproducibility. Other factors being the same, OGT should be a function of the volume of the specific generation apparatus used.

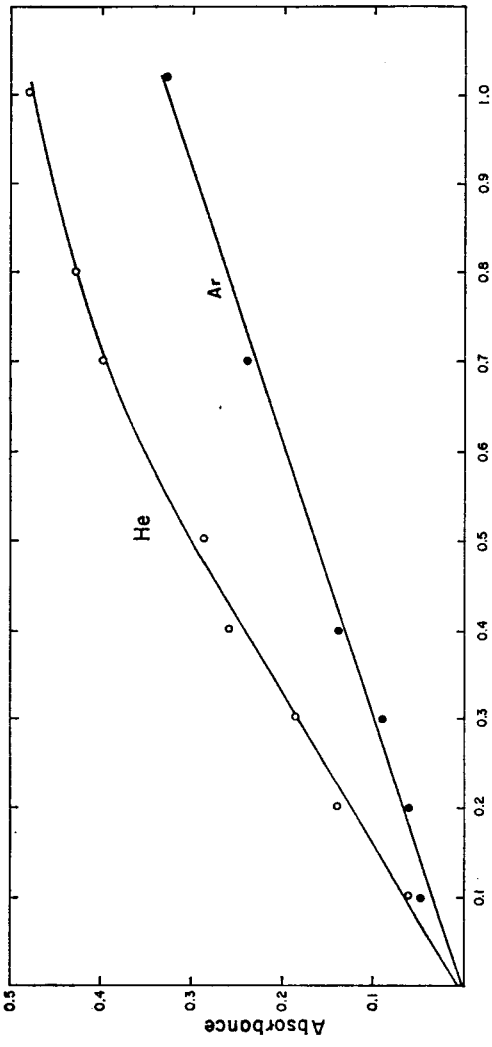

FIGURE 2 Standard calibration curves for two carrier gases (He = helium, Ar = argon).

Under standard operating conditions, the OGT for standards was always close to 12 seconds. However, the OGT for different tissue samples varied from about 7 to 15 seconds and was not predictable. To ensure that sweeping was accomplished at OGT, the tissue samples were run by sight; that is, when the bleed peak was observed to reach its maximum, the generation system was swept. Although standards indicated the method to be sensitive to levels as low as 40 ng, those much below 200 ng may not provide adequate bleed for OGT assessment. In routine analysis of tissue samples at our laboratory, if no bleed peak is observed, the sample is swept at 15 seconds, which is at the end of the plateau region of the sweep curve illustrated in Figure 3. All samples treated in this manner have shown measurable Se.

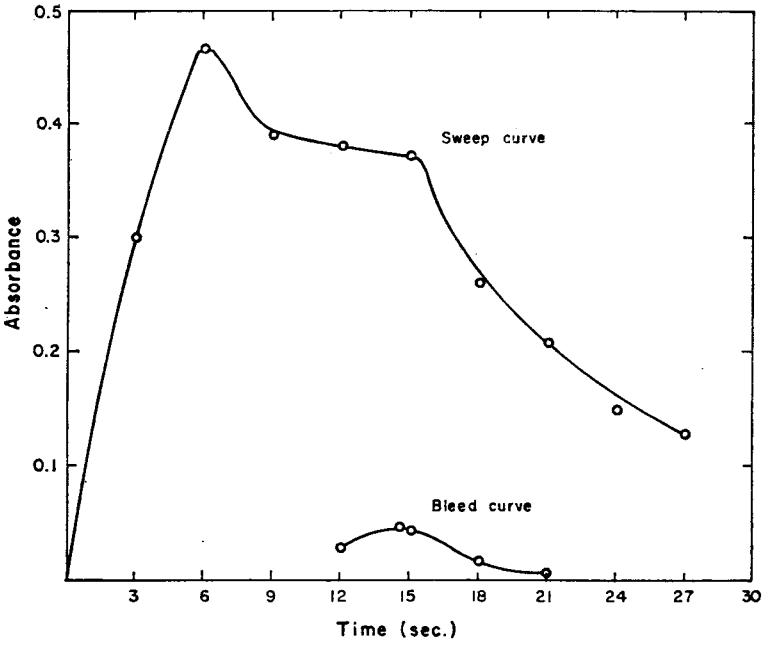

FIGURE 3 Bleed and sweep curves.

It was found that the sweep apparatus absorbed an unpredictable amount of H_2Se unless it was deactivated by pre-saturation with a high level of the gas. When a set of 10 replicate standards containing 1.0 μg Se was run (by sight) before saturation, absorbances ranged from 0.19 to 0.52 with a mean of 0.38 and a relative standard deviation of 30.9%. After a 100-μg standard was swept through the system, the relative standard deviation of standards

improved to less than 10%. Washing the glass joint and Tygon tubing with distilled water was sufficient to de-saturate the apparatus.

A set of 10 replicate standards containing 1.0 μg Se was run by sweeping at the top of an observable bleed peak; absorbances ranged from 0.44 to 0.49 with a mean of 0.47 and a relative standard deviation of 3.6%. Another set of 10 replicate standards containing 0.1 μg Se was run by stopwatch, all at the same OGT as determined by timing the bleed peak of a high-level spike; absorbances ranged from 0.09 to 0.11 with a mean of 0.085 and a relative standard deviation of 9.0%. These two replicate sets could not be compared directly because they contained different Se levels, but each appeared quite reproducible.

To determine the precision and percent recovery of Se organically bound in animal tissue, replicate determinations were made on aliquots of National Bureau of Standards (NBS) Reference Material 1577 (Bovine liver). This certified material contains Se at 1.1 ± 0.1 μg/g as determined by isotope-dilution, spark-source mass spectrometry and neutron-activation analysis. This standard was made into pellets containing 0.33 ± 0.03 μg of selenium and analyzed by our procedure. For 13 runs, recovery ranged from 0.25 to 0.40 μg with a mean of 0.33 μg (100%) and a relative standard deviation of 15%. In comparison, seven 1.0-g samples of homogenized channel catfish tissue were determined to contain 0.29 μg Se per gram with an overall relative standard deviation of 15%.

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