

Table III. Tin in Algae Collected near Scripps Institution of Oceanography

species	ppm, dry wt
<i>Pelagophycus porra</i>	
Pneumatocyst	
pigmented tissue	0.34 ± 0.01
inner tissue	0.03 ± 0.01
blade	0.71 ± 0.01
<i>Macrocystis pyrifera</i> blade	0.83 ± 0.01
<i>Eisenia arborea</i> blade	1.06 ± 0.02

Table IV. Tin in Sediments from Narragansett Bay

depth in core, cm	depositional period ^a	ppm, dry wt ^b
1-2	1972-1973	20
4-5	1969-1970	16
7-8	1966-1967	15
11-12	1962-1963	14
14-15	1959-1960	13
25-27	1935-1947	13
39-41	1900	6
50-53	pre-1900	2
79-84	pre-1900	1

^a Determined by unsupported ²¹⁰Pb (9). ^b Precision of ± 5%.

concentrations of inorganic tin and the butyltin compounds in the upper 20 m may be indicative of atmospheric inputs. It is also possible that the butyltin compounds were derived from the PVC water sampler. The powerful species specificity of the analytical technique, has alerted us to this potential problem and resampling of Lake Michigan waters is underway using polyethylene samplers. As the Lake Michigan waters aged for two months in the sample containers (polyethylene bottles), it was noted that the dibutyltin content decreased and the monobutyltin and Sn(IV) concentrations increased. There was little loss of the organotins to the container.

Sn(IV) removed from seawater collected from the end of the Scripps Institution of Oceanography pier exists in a concentration of 1.5 ± 0.2 ng/L. The values in San Diego Bay

seawater (Table II) are significantly higher and also exceed the concentrations in waters off the California coast.

Tin concentrations in algae and coastal marine sediments from Narragansett Bay are found in Tables III and IV. In these cases, the samples were destroyed by acids, and it is assumed that all tin forms end up as Sn(IV) and that no losses occur during the wet ashing procedure. It is evident from Table IV that tin concentrations in the sedimentary material deposited in the last 50 years in the Narragansett Bay core are significantly higher than those in pre-1900 sediments. This is probably a consequence of the increased uses and subsequent dispersions of tin by man.

In summary, the combination of a hydride generator with atomic absorption spectrometry provides a simple method, with the necessary sensitivity, for determining total tin (in acid digested samples) or Sn(IV) and nine organotin species in environmental samples.

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Atomic Absorption Method for Determining Micromolar Quantities of Thiols

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The quantitative analysis of thiols using atomic absorption spectrophotometry is described. An alcoholic silver nitrate reagent is used to precipitate the silver salts of the respective thiols. These silver salts are then separated from excess reagent, dissolved in nitric acid, and the resultant solution is analyzed for silver concentration using conventional atomic absorption spectrophotometry. One calibration curve was found to be suitable for all of the thiols studied. The relative deviations are 0.8–3.5% in the 0.8–20 $\mu\text{mol/mL}$ range. The solubility product constants of several silver mercaptides were determined by potentiometric titration of the mercaptan with silver nitrate.

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Argentimetric methods for the analysis of thiols are not new to the literature; some of the earliest work dates back to 1929 (1). The titration with silver nitrate has been performed with a variety of visual indicators, including ferric alum and NH_4SCN (1), dithizone (2), and *p*-dimethylamino-benzylidenerrhodanine (3). Potentiometric titrations are frequently plagued with adsorption of excess silver ion on the surface of the precipitate (4). The silver-silver sulfide ion specific electrode has been utilized for the determination of sulfide and cysteine via silver nitrate titration; however, electrode response is sometimes slow (5). Numerous amperometric titrations have been performed on mercaptans and cysteine, but high results are a common problem (6) and electrode poisoning is frequently observed (6). The interferences common to any argentimetric method, namely H_2S

and elemental sulfur, can be removed with cadmium salts and elemental mercury, respectively (1). One mercaptan has been indirectly determined via reaction with silver nitrate and the silver mercaptide formed in the reaction is separated from the reaction medium by filtering and is then digested in concentrated nitric acid. The resultant solution is then analyzed for silver content by means of conventional atomic absorption spectrophotometry (7).

In the present work, thiols are reacted with excess silver nitrate and the silver mercaptide formed in the reaction is separated from the reaction medium by filtering and is then digested in concentrated nitric acid. The resultant solution is then analyzed for silver content by means of conventional atomic absorption spectrophotometry. A single calibration curve is required. In this work, silver nitrate was used to prepare all calibration curves. This procedure allows for the determination of total thiol content of solutions. Pure thiol standards are not required. There are no apparent matrix effects. The analysis is rapid (averaging 15 min per sample) with good sensitivity (0.8–20 μmol mercaptan/mL).

EXPERIMENTAL

Apparatus. Absorbances were measured at 328.1 nm with a Perkin-Elmer 403 atomic absorption spectrophotometer. All filtering was accomplished with a fine frit (4–5 μm) Pyrex glass funnel. A Corning Model 16 expanded-scale pH meter was utilized with a silver wire electrode for all potentiometric titrations. An Orion double-junction reference electrode provided the reference potential.

Reagents. Both the silver nitrate reagent and the thiols were dissolved in isopropanol which had been made aldehyde-free via refluxing with 10% (w/v) NaOH (Aldol condensation). The isopropanol–water azeotrope was distilled and utilized as solvent; the 12% water content facilitated the dissolution of silver nitrate. The silver nitrate reagent at a concentration of 10–100 $\mu\text{mol}/\text{mL}$ was prepared via the appropriate dilution of a 10000 ppm silver nitrate stock solution in the isopropanol azeotrope. Butanethiol, pentanethiol, hexanethiol, heptanethiol, and the three toluene thiol isomers were purchased from the Fisher Scientific Co.; β -mercaptopropionic acid, thioacetic acid, and 2,3-dimercapto-1-propanol were obtained gratis from the Evans Chemetics Co. These thiols and thioacids were used as received following a purity assay via a silver nitrate titration with a potentiometric end-point determination. Benzene thiol was obtained from Eastman Chemicals and was distilled under nitrogen. Silver standards for atomic absorption were prepared via the appropriate dilutions of a solution of 15.7646 g AgNO_3 in 1 L of distilled deionized water. This stock solution was analyzed periodically via a Mohr titration against standard sodium chloride.

Procedure. A micro stirring bar is inserted into a 10-mL beaker. Then 1.00 mL of a solution containing 0.8–20.0 $\mu\text{mol}/\text{mL}$ of thiol in the isopropanol–water azeotrope is pipetted into the beaker. One to two milliliters of a silver nitrate solution (in the azeotrope) is added via Eppendorf pipet with stirring. Within seconds, a yellow or off-white gelatinous precipitate forms. Immediately after addition of the silver nitrate reagent to all of the samples, approximately 2 mL of distilled deionized water is added to each reaction vessel with stirring. After this final addition, stirring is ceased and the samples are allowed to stand at room temperature for 20–30 min. The precipitate is then transferred to a fine sintered glass funnel and filtered with suction. Quantitative transfer is achieved by washing the reaction vessel with less than 10 mL of the wash solution (5–10% v/v NH_4OH in water) and transferring this to the funnel. Then fifteen additional milliliters of wash solution is added in three portions to each beaker and subsequently poured through each funnel. The residual ammonia is removed from each beaker and funnel by washing with 10–15 mL of distilled deionized water. This last wash is also used to wash precipitate from the walls of the funnel onto the frit to facilitate digestion. After this last wash is suctioned off, each funnel was placed above a new clean 125-mL filter flask. Before suction is applied, 20 mL of hot concentrated nitric acid is distributed between each funnel and each beaker and allowed to remain there for 5 min before suction is applied. Suction is

Table I. Analysis of Thiols via Silver Mercaptide Formation

compound	μmol taken	μmol found	recov- ery, %	rel. stand. dev. ^a
benzenethiol	19.78	20.0	101.3	$\pm 3.5\%$ (5)
butanethiol	17.5	18.0	102.4	$\pm 2.6\%$ (3)
pentanethiol	2.05	2.11	103.0	$\pm 2.7\%$ (4)
hexanethiol	1.81	1.85	102.0	$\pm 0.8\%$ (4)
heptanethiol	0.81	0.79	99.4	$\pm 1.6\%$ (4)
<i>o</i> -toluenethiol ^c	5.71	5.78	101.2	$\pm 1.5\%$ (3)
<i>m</i> -toluenethiol	6.79	7.03	103.6	$\pm 2.0\%$ (4)
<i>p</i> -toluenethiol	4.82	4.91	101.9	$\pm 1.0\%$ (4)
2,3-dimercapto-1-propanol	12.1	23.4	96.7 ^b	$\pm 2.1\%$ (5)
β -mercapto-propionic acid	5.63	11.44	101.6 ^b	$\pm 1.3\%$ (4)
thioacetic acid ^c	8.89	18.03	101.4 ^b	$\pm 1.4\%$ (8)

^a Figures in parentheses indicate the number of determinations. ^b Based on 2 mol silver/mol of compound.

^c In the presence of sodium acetate.

then applied to remove all of the dissolved silver ion. The digesting acid in the reaction vessel is passed through the funnel as well. The filter and reaction vessel are rinsed with 20–30 mL of distilled deionized water; as before, the washings from the beaker are passed through the funnel. The contents of the filter flask is then quantitatively transferred to any suitable volumetric flask and diluted to volume with distilled deionized water. The resultant solution is analyzed by atomic absorption spectrophotometry for silver content. If necessary, further dilutions with water may be made to ensure operation in the optimum working range of the atomic absorption spectrophotometer.

Aliquots of the silver stock solution are taken and diluted to volume to provide standards in the 1–6 ppm range. An equivalent amount of nitric acid is added to each standard to compensate for the amount of acid required to digest the precipitate. In addition, an acid blank consistent with the required amount of nitric acid is run through all glassware used in the determination and is diluted to volume with distilled deionized water in the absence of any silver species. (In all cases, the acid blank contributed less than 0.6% to the absorbance of the sample.)

The solubility product constant measurements are made in neutral solution at 25 °C. In order to maintain a constant ionic strength, 1.22 g NaClO_4 is dissolved in 100 mL of the isopropanol–water azeotrope in a 150-mL beaker. One hundred milligrams of thiol is then added from a syringe which is weighed by difference. The initial potential is measured and titration with standard 0.1 N AgNO_3 in the isopropanol–water azeotrope is begun. The potential is recorded throughout the titration after equilibration is attained with each new addition of silver ion. A 50% excess of silver nitrate is added to ensure stable post-equivalence measurements for the last section of the titration curve. End-point potentials are determined graphically. The standard reduction potential for silver ion in the azeotrope is determined by an extrapolation of a plot of potential vs. the log of the apparent silver ion concentration of silver standards prepared in the azeotrope at constant ionic strength. In agreement with the literature, the response is less than Nernstian (55 mV/decade) (8).

RESULTS AND DISCUSSION

The results obtained for eight aliphatic and aromatic thiols, a dithiol, a mercapto acid and a thio acid appear in Table I. Concentration studies were performed on benzenethiol, pentanethiol, hexanethiol, and heptanethiol; a linear response in the range of 0.8–20 $\mu\text{mol}/\text{mL}$ is indicated. In some cases, recoveries tend to be higher at both the lowest and highest concentration levels. This may be explained by adsorption and occlusion of excess silver ion. At lower concentrations, a larger excess of silver ion is needed to drive the reaction to completion, resulting in a larger amount of silver ion available for adsorption and occlusion. At higher thiol concentration, more silver mercaptide is formed, resulting in a larger surface

Table II. Determination of Mixtures of Thiols

	$\mu\text{mol each}$ RSH taken	total μmol RSH taken	total μmol RSH found	recovery, %	rel. std. dev. ^a
mixture 1: pentanethiol	4.80	6.73	6.65	99.0	$\pm 1.8\%$ (8)
hexanethiol	1.93				
mixture 2: <i>o</i> -toluenethiol	1.68	6.50	6.45	99.3	$\pm 3.0\%$ (4)
<i>p</i> -toluenethiol	4.82				
mixture 3: <i>o</i> -toluenethiol	1.68	6.49	6.33	97.6	$\pm 1.0\%$ (4)
<i>m</i> -toluenethiol	4.81				

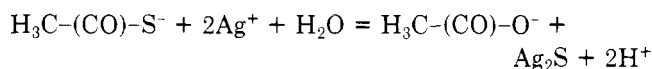
^a Numbers in parentheses indicate number of determinations.

area available for adsorption and occlusion.

Effect of Filter Porosity and Precipitate Form. A medium frit Pyrex glass filter (10–15 μm) was examined and visual loss of precipitate was noted. In an attempt to coagulate some of the more gelatinous precipitates, heat was applied to the reaction vessel after the addition of the silver reagent. This resulted in an aggravation of the problem of high results due to occluded silver ion, so no attempt was made to form larger particles for filtration through faster filters.

Effect on Wash Solution. In order to remove excess silver ion that may be occluded in or adsorbed on the precipitate, the precipitate is washed with a 5–10% v/v NH_4OH solution in distilled deionized water. Washing with 10% sodium thiosulfate, 10% nitric acid, 10% ammonium nitrate, or distilled water resulted in consistently high recoveries, indicating that the excess silver ion was not being removed from the precipitate. An attempt was made to coat the precipitate with nitrobenzene, but this was unsuccessful.

Types of Thiols Analyzed. Simple aliphatic and aromatic thiols react with a one-to-one stoichiometry with silver ion. As expected, 2,3-dimercapto-1-propanol reacted with 2 mol of silver/mol of dimercaptan. β -Mercaptopropionic acid was found to yield a recovery of 1.6 mol silver/mol until sodium acetate was added to the reaction mixture. This weak base favors the dissociation of the carboxylic acid group, allowing for the formation of the silver salt of the acid as well as the silver mercaptide. Since this salt dissolves in dilute ammonium hydroxide, the precipitate was washed with distilled deionized water. Adsorption was not found to be a major problem. Recoveries were quantitative based on 2 mol of silver/mol of mercapto acid. In the presence of sodium acetate, thioacetic acid decomposed to liberate H_2S , so silver sulfide was obtained with the addition of silver ion.



Thus, the molar ratio becomes 2 mol of silver/mol of thioacetic acid.

Analysis of Mixtures. Several mixtures of thiols were analyzed by this method. Results are shown in Table II. The individual thiols of the mixture are not distinguished; the analysis yields the total thiol concentration in solution.

Interferences. Substances that reduce silver ion to silver metal or that form insoluble silver salts may present an interference with this method. However, precipitates that dissolve in dilute ammonium hydroxide solutions (silver oxide, silver chloride, silver cyanide) are removed during the washing procedure. In addition, halides are not often present in the same sample with thiols. Hydrogen sulfide presents a real interference, but literature methods exist for its removal (1). Aldehydes would not undergo the Tollen's reaction under the conditions of the mercaptan precipitation, and unreacted aldehydes may be washed from the silver mercaptide before

Table III. Solubility Products of Silver Mercaptides

parent thiol	pK_{sp}	av. dev.
butanethiol	15.	1
pentanethiol	14.9	0.2
hexanethiol	15.6	0.1
heptanethiol	14.6	0.2
benzenethiol	14.2	0.1
mercaptopropionic acid	13.5	0.1
2,3-dimercapto-1-propanol	20.0	0.1
<i>o</i> -toluenethiol	12.7	0.2
<i>m</i> -toluenethiol	16.2	0.1
<i>p</i> -toluenethiol	14.6	0.1

ammonium hydroxide (which is not a sufficient requirement for aldehyde oxidation via excess silver ion) is introduced.

Calculation of pK_{sp} . For the potentiometric determination of the solubility product of a 1:1 silver mercaptide salt (AgSR), the expression is:

$$E_{\text{e.p.}} = E_{\text{Ag}^+/\text{Ag}}^0 - 2.303 \frac{RT}{2F} \text{pK}_{\text{AgSR}}$$

where $E_{\text{e.p.}}$ = the potential at the equivalence point of the titration, $E_{\text{Ag}^+/\text{Ag}}^0$ = the standard reduction potential for silver ion in the solvent of interest, R = the thermodynamic gas constant, T = the temperature in degrees Kelvin, F = the Faraday constant, 96 500 coulombs/equiv, and pK_{AgSR} = the negative log of the solubility product constant for the silver mercaptide.

The junction potential for the reference electrode was determined to be 10 mV by the measuring of the potential difference between the double-junction reference electrode and a standard calomel reference electrode in isopropanol-water. The extrapolated standard reduction potential for silver ion was corrected for this junction potential and adjusted from standard calomel to standard hydrogen potential. The final value for the reduction potential was 807 mV.

The pK_{sp} values for the silver mercaptides are shown in Table III. Despite the higher solubility, the silver salt of *o*-toluene thiol could be washed with the ammonia wash solution without solubility losses when precipitated in the presence of NaOAc . Butane thiol exhibited strong adsorption of excess silver ion resulting in poor precision during the titration. Attempts to eliminate adsorption by adding NH_4OH drastically changed the potential, as expected, and also contributed to oxidation. The data indicate that a quantitative precipitation analysis can be performed with a dilute ammonia wash solution in the case of every thiol studied with no observable losses due to solubility.

The effect of hydrogen ion concentration on the solubility product constants of the silver mercaptides was not examined for the following reasons.

(1) Basic solutions could not be studied; they changed the electrode potential due to complexation effects and strongly contributed to oxidation of the mercaptan.

(2) Very strong acid solutions digested the organic portion of the silver mercaptide and result in an apparent increase in solubility.

(3) An unsuccessful attempt to utilize dilute solutions of nitric acid to remove adsorbed silver ion from the precipitate resulted in no appreciable solubility losses, indicating a negligible effect of hydrogen ion concentration on solubility.

(4) The general expression for the pH dependence of the solubility (S) of salts of weak acids of dissociation constants (K_a) is given by:

$$S^2 = K_{sp} (1 + [H^+]/K_a)$$

Butler notes that when $[H^+]/K_a$ is small compared to unity (i.e., for solutions of little acidity or for insoluble salts of strong acids), the solubility is independent of hydrogen ion con-

centration (as though no hydrolysis had occurred (9)).

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Alternative Graphical Methods for the Spectrophotometric Analysis of Mixtures

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One method permits data to be taken at multiple wavelengths to generate linear plots, from which the concentrations can be determined; this method is applicable to mixtures of two or three components. The second method is a graphical technique for the solution of simultaneous equations. For 3-component mixtures, the graphical solution is carried out on a triangular composition plot; for 4-component mixtures, a 3-dimensional construction is used. Results from these methods on many mixtures are compared with the conventional simultaneous equations method.

Consider this analogy between reaction kinetics and absorption spectroscopy: in kinetics the data display is essentially a plot of concentration against time; in spectroscopy, absorbance is plotted against wavelength. The formal similarity between the time and wavelength axes suggested that some recently described graphical techniques for the kinetic analysis of mixtures (1, 2) might be usefully applied to spectrophotometric mixture analysis.

PRINCIPLES

Graphical Analysis Using Data at Many Wavelengths. Throughout this paper we assume adherence to Beer's law and additivity of absorbances; let the cell path length be 1.0 cm. Then for the 2-component mixture of A and B, we can write

$$A_t = \epsilon_A c_A + \epsilon_B c_B \quad (1)$$

where A_t is the absorbance of the mixture, and ϵ_i and c_i are the molar absorptivity and molar concentration of species i , the absorbance and absorptivities referring to a common wavelength. To analyze the mixture spectrophotometrically, ϵ_A and ϵ_B must be different functions of wavelength. Then, from Equation 1,

$$\frac{A_t}{\epsilon_A} = c_A + \frac{\epsilon_B}{\epsilon_A} c_B \quad (2)$$

Thus a plot of A_t/ϵ_A vs. ϵ_B/ϵ_A , all quantities evaluated at the same wavelength, is made with data points taken at as many wavelengths as desired. The concentration c_B is obtained from the slope, and c_A can be evaluated by extrapolation to $\epsilon_B/\epsilon_A = 0$; alternatively, the sum $c_A + c_B$ is determined by interpolation at the point where $\epsilon_B/\epsilon_A = 1$. Another way to treat the data is to replot as A_t/ϵ_B vs. ϵ_A/ϵ_B ; then c_A is found from the slope. Yet another means is to extrapolate the line (for Equation 2) to $A_t/\epsilon_A = 0$; the intercept $\epsilon_B/\epsilon_A = -c_A/c_B$.

For a 3-component mixture Equation 3 can be written

$$A_t = \epsilon_A c_A + \epsilon_B c_B + \epsilon_C c_C \quad (3)$$

Define total concentration $c_t = c_A + c_B + c_C$, and fractional compositions $f_A = c_A/c_t$, $f_B = c_B/c_t$, $f_C = c_C/c_t$; then Equation 3 becomes

$$\frac{A_t}{c_t} = \epsilon_A f_A + \epsilon_B f_B + \epsilon_C f_C \quad (4)$$

Note that A_t/c_t can be interpreted as an apparent molar absorptivity for the mixture; i.e., let $A_t/c_t = \epsilon_{app}$. Then upon rearrangement,

$$\frac{\epsilon_{app} - \epsilon_C}{\epsilon_A - \epsilon_C} = f_A + \frac{\epsilon_B - \epsilon_C}{\epsilon_A - \epsilon_C} f_B \quad (5)$$

where we have used the identity $f_A + f_B + f_C = 1$. A plot of the left-hand side against $(\epsilon_B - \epsilon_C)/(\epsilon_A - \epsilon_C)$, over a range of wavelengths, yields the fractions.

For Equation 5 to be useful, the total concentration is needed. This is usually accessible through the following analysis. Suppose two of the components (say A and B) exhibit at least two iso-absorptive points, when the spectra are compared on a molar basis. Thus, at wavelengths where $\epsilon_A = \epsilon_B$, Equation 3 can be written as

$$\frac{A_t}{\epsilon_A} = c_A + c_B + \frac{\epsilon_C}{\epsilon_A} c_C \quad (6)$$

and a plot of A_t/ϵ_A vs. ϵ_C/ϵ_A , using data only at the iso-ab-