6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring silver in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify silver. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect silver in environmental samples are the methods approved by federal agencies such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by a trade association such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

The analytical methods used to quantify silver in biological and environmental samples are summarized in two tables. Applicable analytical methods for determining silver in biological fluids and tissues are listed in Table 6-1, and those used'for determining silver in various environmental samples are listed in Table 6-2.

6.1 BIOLOGICAL MATERIALS

Trace levels (10^{-6} to 10^{-9} g/g of sample) of silver can be accurately determined in biological samples by several different analytical techniques, provided that the analyst is well acquainted with the specific problems associated with the chosen method. These methods include high frequency plasma torch-atomic emission spectroscopy (HFP-AES), neutron activation analysis (NAA)., graphite furnace (flameless) atomic absorption spectroscopy (GFAAS), flame atomic absorption spectroscopy (FAAS), and micro-cup atomic absorption spectroscopy (MCAAS).

Atomic absorption spectroscopy equipped with various atomizers is the best and most prevalent analytical method used to analyze trace amounts of silver in biological tissues and fluids. GFAAS offers high detectability (subnanogram/gram of sample) and requires relatively small samples for analysis of biological tissues (DiVincenzo et al. 1985; Segar and Gilio 1973). Background absorption from sample matrix components can be a problem, but correction using a deuterium continuum light source is adequate if cautiously applied (Segar and Gilio 1973). The detection limit of silver in biological tissues was $2x10^{-5}$ μg/g of sample.

TABLE 6-1. Analytical Methods for Determining Silver in Biological Materials

Sample Matrix	Sample Preparation	Analytical Method	Sample Detection Limit	Accuracy	Reference
Biological tissues	Digest sample with HNO_3; evaporate to dryness; add glacial acetic acid and adjust to pH 3; add ammonium pyrrolidine dithiocarbamate and extract with methyl-isobutyl ketone; heat organic phase to dryness; dissolve residue with HNO_3	GFAAS	0.0012 µg/g (ketone extract) 0.00002 µg/g (extract after reversion to aqueous solution)	No data	Segar and Gilio 1974
Whole blood	Dilute sample with water; agitate in ultrasonic bath and analyze	GFAAS	0.5 µg/100 mL	100-120% recovery	DiVincenzo et al. 1985
	Pipette sample into nickel micro-cup and dry at 150•C	MCAAS	0.27 µg/100 mL	98%-110% recovery	Howlett and Taylor 1978
	Add EDTA solution to sample; dilute sample with triton and ammonium hydrogen phosphate; introduce sample solution into a graphite furnace tube; ash sample at 900•C and atomize at 2,000•C	GFAAS	0.015 µg/100 mL	95%-104.5% recovery	Starkey et al. 1987
	Digest sample with 70% perchloric acid and concentrated HNO_3; evaporate to dryness; add 0.4 M NaI and bismuth solution; heat and analyze	HFP-AES or DCP-AES	0.025 µg/100 mL	90%-110% recovery	Nakashima et al. 1975
	Add EDTA solution to sample; add concentrated HNO_3 and shake vigorously; centrifuge at 5,000 g, separate supernatant and analyze	GFAAS	0.24 µg/100 mL	98% recovery	Vince and Williams 1987
Hair	Wash sample with benzene; filter solution on paper disk and dry disk; insert sample into quartz tube open from both ends; wash sample with water at 50•C and irradiate	NAA	0.69 ppm	No data	Dutkiewicz et al. 1978

TABLE 6-1 (Continued)

Sample Matrix	Sample Preparation	Analytical Method	Sample Detection Limit	Accuracy	Reference
Hair (Cont.)	Wash hair with water and air-dry; digest sample with concentrated HNO_3 by heating; cool sample and dilute to required volume with water	GFAAS	0.02 µg/g	90%-95% recovery	DiVincenzo et al. 1985
Feces	Homogenize sample with water and lyophilize; dissolve ash residue with concentrated H_2SO_4 and HNO_3 and evaporate excess acid to dryness; add HNO_3 and dilute to required volume with water	GFAAS FAAS	0.2 µg/g 3.0 µg/g	80%-100% recovery	DiVincenzo et al. 1985
Liver	Dry sample at 100°C overnight; digest with a mixture of 16 M HNO_3 and 12 M HCl at 100°C; centrifuge and decant supernatant; extract remaining lipid with hot water; cool and recentrifuge; evaporate supernatant to a small volume and dilute with water	FAAS	0.34 µg/g	99%-101% recovery	Johnson 1976
	Ash sample overnight at 450°C with HNO_3; dissolve ash with 50% aqueous HCl; filter sample and analyze at 328.1 nm	AAS	0.0001-0.0005 µg/g	88-92% recovery	Pickston et al. 1983
Pulmonary tissues	Fix tissue sample in 10% buffered formalin for 24 hours; dehydrate in alcohol and embed in paraffin; section sample at 7 microns; stain in hematoxylin and eosin solutions	XES and SEM	Seven-micron-thick sections	No data	Brody et al. 1978
Urine	Evaporate sample to dryness; wet ash residue by heating with concentrated H_2SO_4 and HNO_3 and evaporate excess acid to dryness; add HNO_3 and dilute to required volume with water	GFAAS	0.005 µg/L	110%-130% recovery	DiVincenzo et al. 1985
	Adjust sample to pH 2 with HNO_3 and analyze	GFAAS	1.4 µg/L	99%	Vince and Williams 1987

GFAAS = graphite furnace (flameless) atomic absorption spectroscopy; MCAAS = micro-cup atomic absorption spectroscopy; DCP-AES = direct current plasma-atomic emission spectroscopy; HFP-AES = high frequency plasma-torch-atomic emission spectroscopy; NAA = neutron activation analysis; FAAS = flame atomic absorption spectroscopy; AAS = atomic absorption spectrophotometer; XES = X-ray energy spectrometry; and SEM = scanning electron microscopy.

TABLE 6-2. Analytical Methods for Determining Silver in Environmental Samples

Sample Matrix	Sample Preparation	Analytical Method	Sample Detection Limit	Accuracy	Reference
Simulated solid-waste leaches	Digest sample with a mixture of HNO_3 and HF at 100•C overnight; cool solution and add $HClO_4$; heat until sample is evaporated to dryness; dissolve residue in HCl and water	FAAS	0.568 µg/mL (level 1) 0.473 µg/mL (level 2)	No data	Rains et al. 1984
		DCP-AES	0.53 µg/mL (level 1) 0.38 µg/mL (level 3)	No data	
Rain and stream water	Extract sample with organic solvent; concentrate and analyze atom	GFAAS	ng/mL range	No data	Rattonetti 1974
Fresh water	Add 2% citric acid solution to sample and evaporate solution; add buffer (pH 7.2) and react with succinate dehydrogenase -- chromogenic complex solution	Paper chromatography or micro TLC	1 µg/sample	No data	Devi and Kumar 1981
Commercial condensed milk	Digest sample with 70% perchloric acid and concentrated HNO_3 solution, evaporate solution to almost dryness; dissolve residue in water and add 0.4 M NaI and bismuth solution; heat and analyze	HFP-AES or DCP-AES		89%-94% recovery	Nakashima et al. 1975
Air	Collect sample through a Delbag Mikrosorban filter or General Electric filter; store sample in sealed polyethylene bag; irradiate sample and analyze	NAA	0.13 µg/10 cm^2 (Delbag Mikrosorban filter); 0.008 µg/10 cm^2 (General Electric filter)	No data	Bogen 1973
	Calibrate sampling pump; collect sample at known flow rate; add concentrated HNO_3 and $HClO_4$; heat sample solution to dryness; dissolve residue ash in 4% HNO_3 and 1% $HClO_4$ solution; analyze at 328.3 nm	ICP-AES	26 ng/mL	91%-111% recovery	NIOSH 1984b (method 7300)

TABLE 6-2 (Continued)

Sample Matrix	Sample Preparation	Analytical Method	Sample Detection Limit	Accuracy	Reference
	Collect sample at rate of 20 liter/min using acetyl-cellulose filter and analyze at 328 nm	AAS	$3x10^{-4}$ mg/mL	No data	Soldatenkova and Smirnov 1983
	Filter particulate matter from air; irradiate and count sample	NAA (nondestructive)	0.1 μg/ sample	No data	Dams et al. 1970
Raw beef	Prepare ash of sample by heating to 500°C; hydrolyze ash sample with 6 N H_2SO_4 and adjust pH to 1.8-2.0; add 2 N ammonium acetate solution and stir overnight; centrifuge and analyze	GSE	0.013 ppm	No data	Mitteldorf and Landon 1952
Waste water	Digest sample and add 5% potassium citrate, phenolphthalein indicator, and 4 M NaOH until solution turns red; add HNO_3 to decolorize solution; finally add buffer (pH 5), 0.1 M EDTA, 1% sodium lauryl sulfate and 0.5 m/g (3,5-diBr-PADAP) in ethanol; measure absorbance at 570 nm	UV	0.39 ppm	>90% recovery	Hung et al. 1982
Metallic silver	Add 0.3 N HNO_3 to sample and adjust to pH 2.3; extract sample with an automated extraction system	FAAS	0.4 μg/L	No data	Pierce et al. 1975
Eye lotion	Add silver nitrate sample to 95% HNO_3 solution and heat to 80-90°C while agitating; cool and filter solution; react filtrate by shaking with a solution of 0.2% dithizone in chloroform; analyze silver in silver nitrate solution at 400 nm	PD	50 ppm	4% error	Massa 1969

FAAS = flame atomic absorption spectroscopy; DCP-AES = direct current plasma-atomic emission spectroscopy; GFAAS = graphite furnace (flameless) atomic absorption spectroscopy; TLC = thin layer chromatography; HFP-AES = high frequency plasma-atomic emission spectroscopy; NAA = neutron atomic analysis; ICP-AES = inductively coupled plasma-atomic emission spectroscopy; AAS = atomic absorption spectrometry; GSE = graphite spectroscopic electrode; UV = ultraviolet spectrophotometry; PD = photodensitometer; and (3,5-diBr-PADAP) = 2(-3,5-dibromo-2-pyridylazo)-5-diethyl-aminophenol.

Recently, Starkey et al. (1987) modified the GFAAS technique for determining trace levels of silver in the blood of exposed and unexposed individuals. Ethylene diamine tetraacetic acid (anticoagulant) and ammonium hydrogen phosphate buffer (matrix modifier) were added to blood samples prior to analysis. Starkey and co-workers indicated that the GFAAS technique is highly selective and sensitive and does not require a complex sample pretreatment (ashing and digestion with strong acids). A detection limit of 15×10^{-3} μg/100 mL of sample was reported.

Howlett and Taylor (1978) used an atomic absorption spectroscopy fitted with a micro-cup assembly (MCAAS) for determining silver levels in human whole blood. The MCAAS technique affords a rapid, precise, and relatively simple method for the measurement of silver in blood. Furthermore, this technique requires no sample preparation prior to analysis except pipetting and drying. A detection limit level of 0.27 μg/100 mL of blood sample was measured. Howlett and Taylor (1978) noted that repeated measurement of silver in blood using a single nickel cup showed a gradual decrease in sensitivity.

FAAS technique has been successfully used to detect levels of silver in post-mortem human liver; the detection limit for this method was 0.34 μg/g (Johnson 1976).

HFP-AES can determine ng amounts of silver in a small sample of human blood. Prepared human blood sample was introduced into the atomizer chamber as an aerosol, formed by nebulization of the sample solution (Nakashima et al. 1975). The authors noted that the sensitivity of the HFP-AES technique was improved by eliminating moisture in the aerosol with a second condenser at -3 to -5°C. The use of bismuth as a coprecipitate showed an enhancing effect on the silver emission at 328.06 nm. A detection limit of 0.25 μg/100 mL of sample was attainable. Advantages of the HFP-AES methodology include freedom from most types of chemical interference, high sensitivity, and multielemental capability. However, this technology might have to be adapted to currently available instrumentation in order to be useful. The presence of spectral interferences is a disadvantage of plasma emission spectroscopy. These interferences are caused when a sample contains elements that have analytical emission lines that overlap the line chosen for the analyte. Blood is particularly troublesome because of high concentration of iron. Iron has a very complex emission spectrum. Also, the analytical line for silver used in the Nakashima et al. paper has interference from manganese. For this reason, the blood is subjected to dangerous perchloric acid/nitric acid digestion and preconcentration of silver ion prior to analysis. Other inherent disadvantages of HFP-AES include the employment of time-consuming procedure, the need for standard additions for accurate quantification, and its high costs when compared to GFAAS. Unless a laboratory is already furnished with the instrumentation, purchase of HFP-AES is not recommended for the analysis of silver alone. GFAAS or even DCP-AES could be employed for the determination of silver in biological samples.

6. ANALYTICAL METHODS

Owing to its high sensitivity, the NAA technique has been widely employed for determination of trace elements (including silver) in biological and environmental samples. The NAA technique is based on interaction of the nuclei of individual silver atoms of the sample with neutron irradiation, resulting in the emission of γ-rays (photons). The radioactivity of the irradiated sample is measured with a high-resolution lithium-drifted germanium detector. The long-lived, metastable ^{110m}Ag isotope of silver was formed following irradiation of human hair samples. A half-life of 250.4 days for ^{110m}Ag gives ample time to initiate counting after an irradiation and cooling period (Dutkiewicz et al. 1978). The authors noted a detection limit for silver of 0.69 ppm in human hair. (See Section 2.5 for a discussion of the disadvantages of using hair samples for monitoring exposure to silver.) A disadvantage of NAA is that it is a very expensive technique and may not be readily available in most laboratories.

DiVincenzo et al. (1985) employed the GFAAS technique to evaluate human samples for biological monitoring of silver exposure levels in the workplace. The authors determined the total silver concentration in urine, blood, feces, and hair with detection limits of 0.005 μg/L,0.5 μg/100 mL, 0.2 μg/g, and 0.02 μg/g, respectively.

Scanning electron microscopy (SEM) in concert with x-ray energy spectrometry (XES) has been used to detect silver in pulmonary, lacrimal sac, and skin tissues of individuals with diffuse interstitial lung disease, chronic dacryocystitis, and skin disorders, respectively (Brody et al. 1978; Loeffler and Lee 1987; Tanita et al. 1985). Brody et al. (1978) observed particles of preselected lesions of human pulmonary tissue magnified to 300x by SEM, and the silver content was analyzed by XES. The authors noted that SEM and XES techniques permit a rapid and conclusive determination of silver, silver compounds, and complexes in tissue lesions.

6.2 ENVIRONMENTAL SAMPLES

Atomic absorption and plasma emission spectroscopy are perhaps the most widely used analytical techniques for the determination of silver levels in air, soil, and water. Rains et al. (1984) employed atomic absorption spectroscopy with flame atomization (FAAS) and direct current plasma-atomic emission spectroscopy (DCP-AES) to determine silver levels in solid-waste leachate. In the FAAS technique, a diluted solution of the sample following ashing and digestion is sprayed into a flame by means of a nebulizer. The high temperature causes formation of atoms, which can be observed (at 328.1 nm resonance line) by absorption spectroscopy. The authors noted that interference encountered by the FAAS technique was largely alleviated by the use of 1% solution of ammonium dibasic phosphate buffer as a matrix modifier. In the DCP-AES technique, Rains and co-workers observed silver as a broad band emission at 328.068 nm resonance line. Addition of lithium carbonate to sample solution

reduces the inter-element interferences observed in unbuffered direct-current plasmas, but does not significantly degrade DCP-AES detection limits. Detection limits of silver in solid-waste leachate sample by FAAS and DCP-AES techniques were 0.473×10^{-6} g/mL sample and 0.38×10^{-6} g/L sample, respectively (Rains et al. 1984).

GFAAS technique is more sensitive than FAAS methodology for determination of silver in water samples. Rain and stream water have been analyzed by GFAAS technique to detect silver at ng/mL levels (Rattonetti 1974).

Inductively coupled argon plasma with atomic emission spectroscopy (ICP-AES) has been recommended by NIOSH (method 7300) for determining silver in air. ICP-AES offers multi-element capabilities and high sensitivity but spectral (background) interference can be a problem (NIOSH 1984b). The EPAestablished analytical test procedure (method 200.7) to analyze dissolved, suspended or total silver in drinking water, surface water, and domestic and industrial wastewaters employs the ICP-AES technique (EPA 1987a). An estimated detection limit of 7.0×10^{-6} g silver/L sample was measured.

Neutron activation analysis (NAA) methodology has been used to determine silver levels in environmental samples. Bogen (1973) reported a detection limit of 8×10^{-9} g silver/l0 cm^2 filter. The author indicated that the use of high-resolution lithium-drifted-germanium detection allows multi-elemental analysis to be performed in a single measurement without any chemical pretreatment of the air sample. A highly precise, sensitive, and nondestructive computer-assisted NAA technique for the determination in air of multi-element particulate matter has been designed by Dams et al. (1970). The authors reported a detection limit of 1×10^{-7} g silver/sample. The NAA technique by Bogen (1973) and Dams et al. (1970), utilizes the long-lived isotope of silver (^{110m}Ag) for quantifying silver levels in air. The faster nondestructive NAA technique developed by Dams et al. (1970) utilizes the short-lived isotope ^{110}Ag (half-life = 24.6 seconds) to detect silver in air following an 18-second neutron irradiation of air sample. Hence, counting can be initiated after an irradiation and cooling period of a few minutes.

Hung et al. (1982) developed a sensitive and selective method for silver analysis by reacting silver (I) with 2(-3,5-dibromo-2-pyridylazo)-5-diethyl amino phenol in the presence of an anionic surfactant, sodium lauryl sulfate. The ternary complex formed is red and exhibits an absorption peak at 570 nm. Hung and his co-workers employed EDTA as a chelating agent, thereby reducing the interference of common ions. Recoveries were good, and a detection limit of 0.39 ppm of silver was achieved.

Paper chromatographic, micro thin-layer chromatographic (TLC) and photodensitometric (PD) methods have also been successfully used to determine levels of silver compounds in freshwater and eye lotion samples (Devi and Kumar 1981; Massa 1969). Simple paper and micro thin layer chromatographic (TLC) techniques were employed by Devi and Kumar (1981) to detect and quantify

trace (40 ppm) levels of silver nitrate in fresh water. Devi and Kumar reacted a prepared silver nitrate sample with succinate dehydrogenase enzymechromogenic reagent complex solution prior to paper chromatographic or micro TLC analysis. The metals are recognized by their ability to inhibit the enzymatic formation of a pink reaction product.

Soil samples have been analyzed for silver by AAS (Klein 1972), NAA, and x-ray fluorescence analysis (Ragaini et al. 1977). No statements on the sensitivity, accuracy, or precision of these methods for soil analysis were presented in the brief description of these methods.

6.3 ADEQUACY OF THE DATABASE

Section 104 (i) (5) of CERCLA, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of silver is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of silver.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met would reduce or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Existing methods of measuring levels of silver in blood, urine, feces, hair, and tissues are extremely sensitive and can measure levels in the low ppm to ppt. These methods are accurate and reliable and can be used to measure both background levels of exposure and levels at which biological effects occur. No additional analytical methods for determining trace levels of silver in biological materials are needed.

Highly sensitive methods exist to measure silver concentrations in blood, urine, hair, and skin samples of individuals showing the few health effects that have been associated with silver exposure. These methods are also able to accurately measure background levels in the population. No additional analytical methods appear to be needed for the known biomarkers of effect.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Sophisticated and highly refined methods are available

to detect trace levels of silver and its compounds in air, solid waste leachate, water (the medium of most concern for human exposure), food, and other environmental media. These methods can accurately measure background levels in environmental samples, as well as levels at which health effects occur. There are no known deficiencies in the analytical methods for determining silver in environmental media, and no additional analytical methods appear to be necessary.

6.3.2 On-Going Studies

No on-going studies concerning techniques for measuring and determining silver in biological and environmental samples were located.