THE DETERMINATION OF SILVER IN WHOLE BLOOD AND ITS APPLICATION TO BIOLOGICAL MONITORING OF OCCUPATIONALLY EXPOSED GROUPS

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Abstract-A sensitive and rapid technique for directly measuring silver in blood, using electrothermal atomization atomic absorption spectrophotometry (ETAAS) is described. The method can be used to analyse precisely up to 40 blood samples a day in duplicate.

Well-mixed, whole blood samples, collected in EDTA, were diluted 1+4 with a diluent containing 40 g l.~' ammonium dihydrogen orthophosphate and 0.5 ml l.~' Triton X-100. Aliquots of diluted blood were then analysed by ETAAS using wall atomization with a pyrolytically coated tube.

The coefficient of variation for within-run precision was 4.55% at 10 μg l.~' and 5% at 25 μg l.~'. Between-run variation, it was 4.1% at 25 μg l.~'. The analytical recovery for the method was 98% ±3% at both 8 and 30 μg l.~'. The detection limit of the method was 0.1 μg l.~', which was sufficiently sensitive to distinguish exposed from non-exposed individuals. Blood silver levels in unexposed subjects were found to be between <0.1 and 0.2 μg l.~'.

Blood silver levels were determined in 98 occupationally exposed workers involved in bullion production, cutlery manufacture, chemical manufacture, jewellery production and silver reclamation. Blood silver levels ranged from 0.1 to 23 μg l.~', with some of the highest levels found in silver reclaimers.

INTRODUCTION

Silver is used widely in the production of coins, jewellery, tableware and alloys, and in the manufacture of electrical apparatus, mirrors and chemicals for photographic processes. Additionally, it is used in the production of dental amalgams and burn creams, due to its antibacterial properties (Vince and Williams, 1987). It is a non-essential element to man (Fowler and Nordberg, 1986).

The occupational exposure standards (OES) for metallic silver and its compounds are currently 0.1 and 0.01 mg m~3, respectively. Occupational exposure to silver can occur by inhalation or ingestion (Phalen and Morrow, 1973; Furchner et al., 1987). Inhaled silver is deposited in the lung and can be absorbed through the wall of the alveolus. Some of the inhaled particles will be cleared from the lungs by mucocilliary action and swallowed. About 10–20% of the ingested metal is absorbed in the gastrointestinal tract, mainly by the duodenum and small intestine. Gastric hydrochloric acid may convert ionic or metallic silver into the chloride form, which is absorbed more readily (Smith and Carson, 1977). Skin absorption has also been...
reported, but may rely on damage to the epithelium being present (Boosalis et al., 1987).

Silver is distributed around the body and is deposited mainly in the liver, kidney and, to a lesser extent, the spleen. Chronic exposure can lead to irreversible deposition in subcutaneous tissue and mucous membranes. Such deposition and the enhanced melanin production, stimulated by the presence of silver, results in the condition known as argyria (Green and Su, 1987).

The main route of excretion is through the faeces Fowler and Nordberg, 1986). In vivo studies conducted in mice, rats, monkeys and dogs have found more than 90% of an administered dose of silver is excreted via the faeces within 2–4 days of administration (Furchner et al., 1987).

Elimination is biphasic in man. Whole body measurement following accidental inhalation of silver (Newton and Holmes, 1966) showed the half-life of the first phase to be approximately 1 day. This corresponds to faecal clearance of unabsorbed metal particles. The second phase has a half-life of approximately 50 days and reflects elimination from the blood stream, via the liver, into the gut. This half-life suggests that the timing of blood samples, for biological monitoring of occupational exposure, is not critical. Relatively little silver is excreted in urine (Phalen and Morrow, 1973) and will only be of significance if the exposure is high.

Several biological monitoring methods have been considered and described for measuring uptake and excretion of silver (Julshamn et al., 1986; Starkey et al., 1987; Vince and Williams, 1987; Wan et al., 1991). Urine analysis is only of relevance if exposure has been very high (Wan et al., 1991). Methods which involved the analysis of faeces were also discounted due to the impracticability of sample collection. Published methods describing the determination of silver in blood use a variety of instrumentation and rely on strong matrix modification to enable accurate detection. Our aim was to produce a sensitive analytical technique using simple instrumentation and sample preparation which is amenable to automation. Whole blood was chosen as the matrix of measurement for silver as it has been suggested that there may be an ill-defined association between the metal and red blood cells (Jongerius and Jongeneelen, 1992). Thus the possibility exists that, if serum analysis was used, falsely low silver levels may be measured due to silver binding to the red cell fraction.

In this paper, the determination of silver in whole blood is described, using a method which is robust and suitable for monitoring industrial populations. The method has been used to assess the uptake of silver in a control population and in industrial workers, and the results are presented as indication values for various occupational groups.

METHODOLOGY

Apparatus

A Perkin–Elmer model 5100 pc atomic absorption spectrophotometer with a Perkin–Elmer HGA-600 graphite furnace was used. Samples were introduced into the furnace using a Perkin–Elmer AS-60 autosampler. Absorbance data were recorded remotely and analysed using computer software.
The determination of silver in whole blood

Perkin-Elmer pyrolytically coated tubes were used and they were conditioned by firing them six times with the quality control (QC) sample. A sonicator was used to treat the bovine blood used for the preparation of calibration standards. The standards and samples were prepared using calibrated automatic pipettes, grade A glass bulb pipettes and a programmable dispenser/diluter. All glassware used was grade A and it was conditioned prior to use by soaking overnight in 10% nitric acid (Fisons Primar grade) and rinsing twice in deionized double-distilled water, before use.

Reagents

A 1 mg l.\(^{-1}\) working standard was prepared by dilution of a silver standard stock solution (1000 \(\mu\)g ml\(^{-1}\), BDH Ltd, Spectrosol grade). Appropriate volumes of this working solution were further diluted with 50 ml of sonicated bovine blood to give a calibration series of 0, 1.0, 2.5, 5.0, 10.0, 20.0 and 40.0 \(\mu\)g l.\(^{-1}\) added silver. A 0 \(\mu\)g l.\(^{-1}\) standard was included to allow the data handling system to compensate for endogenous silver. A further 50 ml aliquot of blood was spiked to a concentration of 25 \(\mu\)g l.\(^{-1}\) added silver. This was used as an internal QC material, as no commercial QC material was available. One millilitre aliquots of the standards and QC material were dispensed into plastic vials and stored at \(-20^\circ\)C (long term).

A fresh set of standards was thawed and mixed on each day analyses were performed. The diluent was also prepared fresh daily. Four grammes of ammonium dihydrogen orthophosphate (BDH Ltd) were weighed out on the top pan balance, dissolved in deionized double-distilled water (prepared by a Fi-stream still system), to which was added 50 \(\mu\)l of Triton X-100 (BDH Ltd). The solution was made up to 100 ml in a grade A volumetric flask.

Analytical procedure

Initial studies were undertaken to optimize instrument parameters and furnace conditions for the analytical procedure; these are detailed in Tables 1 and 2. The ash temperature chosen was 650°C. At this temperature there was negligible analyte loss and the highly proteinaceous matrix was completely removed by the addition of oxygen to the ashing step. Previous studies on manganese have shown the addition of oxygen to be a valuable aid in the ashing of biological specimens (Shuttler and Delves, 1988). The atomization temperature chosen was 1800°C. At this temperature the absorbance signal had reached a plateau and gave a high signal to background ratio.

The furnace was used in the maximum power heating mode and standards were matrix matched to the samples to compensate for any residual matrix interference effects.

All standards, QC material and samples were diluted 1 + 4 with the ammonium dihydrogen orthophosphate diluent into plastic vials using a Hamilton programmable dispenser/diluter. The vials were capped and mixed and the solution was then transferred to acid-washed autosampler cups. QC samples were run after the calibration curve and after every six samples.
Table 1. Instrument parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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</thead>
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</tr>
<tr>
<td>Bandwidth</td>
<td>0.7 (low)</td>
</tr>
<tr>
<td>Lamp current</td>
<td>10 mA</td>
</tr>
<tr>
<td>Sample volume</td>
<td>15 µl</td>
</tr>
<tr>
<td>Integration</td>
<td>3 s</td>
</tr>
<tr>
<td>Recorder</td>
<td>10 mV</td>
</tr>
<tr>
<td>Background correction</td>
<td>On</td>
</tr>
<tr>
<td>Measurement mode</td>
<td>Peak height</td>
</tr>
</tbody>
</table>

Table 2. Furnace parameters

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<th>Ramp (s)</th>
<th>Hold (s)</th>
<th>Read</th>
<th>Record</th>
<th>Argon (ml min⁻¹)</th>
<th>Oxygen (ml min⁻¹)</th>
<th>Baseline</th>
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</thead>
<tbody>
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<td>300</td>
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<td></td>
</tr>
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<td>20</td>
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<td></td>
</tr>
<tr>
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<td>650</td>
<td>5</td>
<td>30</td>
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<td>On</td>
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<tr>
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<td>1800</td>
<td>0</td>
<td>3</td>
<td>On</td>
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</tr>
<tr>
<td>5</td>
<td>2650</td>
<td>1</td>
<td>3</td>
<td>On</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

Precision and sensitivity

Calibration curves for silver were linear across the range of 0–40 µg l⁻¹. The sensitivity of the method, defined as the amount of silver needed to give an absorbance of 0.0044 absorbance units, was 0.3 µg l⁻¹. The detection limit of the method, defined as three times the standard deviation of the matrix blank, was 0.1 µg l⁻¹. The coefficient of variance for within-run precision was 4.6% at 10 µg l⁻¹ (N=24) and 5.0% at 25 µg l⁻¹ (N=48). Between-run precision was 4.1% at 25 µg l⁻¹ (N=10).

The mean recovery of silver from samples spiked with 8 and 30 µg l⁻¹ silver was determined by analysing each sample 20 times. The 8 and 30 µg l⁻¹ spiked samples gave recoveries of 98% with a standard deviation of ±3%, as did the 30 µg l⁻¹ spiked sample.

Comparison with another laboratory, using a different method for measuring silver, gave a correlation of r=0.98 for 20 blood samples.

Analysis of blood samples

A total of 98 blood samples from occupationally exposed workers and 15 control blood samples from agricultural workers, who were not occupationally exposed to silver, was analysed for silver. Samples were collected on routine surveillance visits by HSE staff or by company doctors at the six factories concerned. The factories were involved with silver reclamation, jewellery manufacture, bullion production, silver chemical manufacture and the production of tableware.

RESULTS

A normal reference range for silver in blood was determined for a population of agricultural workers, who were not occupationally exposed to silver. The mean silver
in blood level of this population was <0.1 µg l.⁻¹ with only four of the 15 samples analysed having a detectable silver concentration (0.2 µg l.⁻¹).

The method was applied to the study of silver exposed workers employed at six different sites and involved in various tasks within each workplace. The data showing the results by factory and job description/code are displayed graphically in Figs 1 and 2.

Reclamation workers (Factory A) were found to have some of the highest silver in blood levels measured, the mean was 6.8 µg l.⁻¹ and the range was 1.3–20 µg l.⁻¹ (N=19). Furnace (Job Code 1) and refinery operators (Job Code 2) were the occupations with the highest potential for exposure to silver (Fig. 2).

Analysis of blood from workers involved in refining silver to produce bullion, coins and chemicals (Factories B, D, E and F) gave a mean silver in blood level of 2.5 µg l.⁻¹, with values ranging between 0.1 and 16 µg l.⁻¹ (N=70). Numerous varied workplace activities were defined in this industry, but melters, refiners and workers involved in the production of silver nitrate (Job Codes 2, 4 and 17, respectively) were the occupation in which the highest silver in blood levels were observed. Workers involved in jewellery production (Factory C) had one of the lowest mean silver blood levels for an occupationally exposed group at 1.2 µg l.⁻¹. The range was 0.2–2.8 µg l.⁻¹ (N=9).

DISCUSSION

The method for silver determination described here is sensitive and robust for biological monitoring purposes and has some advantages over methods published previously. Sample preparation is simple, the furnace programme is short and the
Fig. 2. Blood silver levels found in occupationally exposed workers categorized according to job type. Key: 1, furnace operator; 2, refinery operator; 3, annealer; 4, melter; 5, mill operator; 6, shift supervisor; 7, maintenance; 8, contractor; 9, laboratory sampler; 10, pulverizer; 11, coin polisher; 12, water atomizer; 13, bullion former; 14, luxury department; 15, jeweller; 16, lupel operator; 17, silver nitrate production.

instrumentation is fully automated, allowing a daily throughput of 40 samples in duplicate, with minimal operator intervention. The detection limit and sensitivity of the method allow a distinction to be drawn between occupationally exposed and unexposed workers.

The blood silver concentrations found in occupationally unexposed workers is consistent with that previously reported in the literature (DiVincenzo et al., 1985; Minioa et al., 1990). The blood levels found in the occupationally exposed workers ranged from 0.1 to 20 µg l.−1 (Fig. 1) with no evidence of argyria in any of the workers. These results agree with previously reported blood levels of 6–26 µg l.−1 for workers refining silver to produce chemicals for photographic processes and who showed no evidence of argyria (DiVincenzo et al., 1985). Their study showed faecal elimination of the metal was 0.3 mg per day which they equated to a time-weighted average (TWA) workplace exposure of 0.03 mg m.−3. DiVincenzo et al. (1985) measured static and personal air levels of silver (8-h time-weighted average) over a 2 month monitoring period. Levels detected ranged between 0.001 and 0.1 mg m.−3 and insoluble forms of the metal were considered to be the primary form present.

Of the workers involved in our study, the highest levels were seen in those involved in reclaiming silver (1.3–20 µg l.−1). The furnace operator had a level of 14 µg l.−1. The workers dealing with concentrated silver solutions also had high levels (12 and 20 µg l.−1). Operatives involved in similar processes during bullion production or refining had blood levels approaching those found in reclaimers (0.9–16 µg l.−1). Pifer et al. (1989) estimated airborne levels of 0.005–0.02 mg m.−3 for an 8-h TWA for workers involved in silver recovery from spent photographic chemicals and found an average blood level of 10 µg l.−1, with 21% of subjects having ocular
argyrosis but without visual impairment. Minoia et al. (1985) found very high airborne levels using personal sampling methods for jewellers involved in casting molten silver (0.26–0.6 mg m⁻³). However, the jewellery producers involved in our study were the group with the lowest blood silver concentrations (0.2–2.8 μg l⁻¹). In this case, the workers were producing small, intricate items. The airborne levels of silver, produced when heat is applied to the workpiece, will be significantly less than those found in the heavier industries of silver reclamation, refining and bullion casting. Exposure would, therefore, be anticipated to be lower in small-scale jewellery manufacturers than in large-scale industries.

The levels of silver in blood found for various jobs in the six factories studied are shown in Fig. 2. A wide range of levels was seen within a single job type. This variation can be partly explained by two factors. The efficiency of workplace control will significantly effect the level of exposure seen in an individual as will the personal hygiene of a worker. The immediate exposure history of the individual workers will also influence their results.

Most data on acute exposure to silver are derived from burns patients who are treated with antibacterial silver sulphadiazine cream (SSD). Wan et al. (1991), showed that serum silver levels rise in the period following treatment, reaching peak levels, of up to 310 μg l⁻¹, 3 weeks after initial exposure. The level of exposure in this particular group of patients was extremely high and this may have a bearing on the time taken for maximal values to be obtained. The post mortem examination of tissue samples from one patient, who died of renal failure after 8 days of treatment with SSD cream, showed deposition of silver in the cornea (970 μg g⁻¹ wet tissue), liver (14 μg g⁻¹ wet tissue) and kidney (0.2 μg g⁻¹ wet tissue). Argyria is commonly seen in burns patients treated with SSD cream as the damaged epithelial tissue will allow rapid and extensive adsorption of silver, which is then deposited at various sites.

The amount of silver that will produce the clinical manifestations of generalized argyria depends on the solubility of the silver compound and the route of entry into the body (Greene and Su, 1987). Although exposure conditions giving rise to the condition have not been well defined (Fowler and Nordberg, 1986), it has been suggested that a total dose of 1–8 g of silver would be required to induce the condition following long-term inhalation exposure (Hill and Pillsbury, 1939). Lehnert (1973) stated that the dose required to induce argyria by ingestion was higher, at 1–30 g of soluble silver salts.

The long-term effects of argyria are not detrimental to health. The consequences seem to be more social or psychological, as the pigmentation can cause embarrassment (Green and Su, 1987).

The biological monitoring data collected for occupationally exposed silver workers are of value both on an individual and a group basis. Individually, it can be of use in assessing workplace exposure, in studying work practice, the effectiveness of personal protection and the degree of personal hygiene associated with a particular worker. Indirectly, it may be used to assess the efficiency of local exhaust ventilation and other control procedures associated with an individual's work operations. On a group level, the data can be used to evaluate the overall effectiveness of workplace control and highlight any particular processes or work practices which may warrant further investigation.
REFERENCES


