

Dispersive liquid–liquid microextraction using ammonium *O,O*-diethyl dithiophosphate (DDTP) as chelating agent and graphite furnace atomic absorption spectrometry for the determination of silver in biological samples

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A new method for the determination of silver in biological samples is presented in this work. The method involves application of dispersive liquid–liquid microextraction (DLLME) employing ammonium *O,O*-diethyl dithiophosphate (DDTP) as the chelating agent for extraction and preconcentration of silver prior to quantification using graphite furnace atomic absorption spectrometry (GFAAS). Chloroform and acetone were selected as the extracting and dispersing solvent, respectively, at optimized volumes of 80 μL and 500 μL , respectively. The concentration of DDTP and the extraction time were optimized as 0.01% (m/v) and 10 min, respectively. Pyrolysis (1100 $^{\circ}\text{C}$) and atomization (1800 $^{\circ}\text{C}$) temperatures were optimized using a L'vov platform treated with 400 μg of tungsten as a permanent chemical modifier. The method was proven virtually free from interference from major constituents of biological samples. A detection limit of 2 ng g^{-1} was obtained with relative standard deviations better than 13% and an enhancement factor of 70 was achieved. The determined concentrations for Ag in certified reference biological samples were in good agreement with the certified values at a 95% statistical confidence limit. The reported method using DLLME and GFAAS presented good analytical performance for Ag determination when compared to other methods available in the literature.

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Introduction

Silver is a metal frequently found as a mineral ore in association with other elements. About 2.5 million kilograms of silver are released every year in the biosphere, mostly originating from anthropogenic activities. Silver is extensively used in the manufacture of coins, in electrical products, photographic manipulation, in batteries and in jewels, among other products.¹ The ionic form of silver is well-known as one of the most toxic species for aquatic organisms.² However, major releases of Ag from industry to the aquatic environment are not efficiently monitored, due to the costs associated with this process. Environmental contamination by silver is usually a concern to invertebrates and plants, since the element has only

limited toxicity and its effects are not fully understood in humans and other vertebrates, although bioaccumulation may be a concern for health issues.² Furthermore, the use of silver nanoparticles in diverse products such as clothes and wound dressings due its antibacterial behavior could act as a potential source of contamination, affecting the health status in humans.³

Despite the evident progress in instrumental analysis in the past few decades, many of the current analytical instruments fail to achieve the sensitivity and the freedom from interferences required to the successful determination of trace elements. The growing interest in the study of the effects of trace elements in the environment demanded rigid regulations in the world with respect to what should be assumed as 'safe' concentrations of trace elements in samples such as water, soil and seafood. However, in order to comply with the rigid demands for elemental analysis, mainly with regard to sensitivity, a preconcentration stage is frequently necessary to reach the lowest possible detection limits.⁴ The extraction and preconcentration steps require special attention, because these steps are frequently time-consuming and susceptible to contamination and losses of the species of interest during handling of the sample.⁵

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Trace levels of Ag may be determined using a potentiometric sensor,⁶ an optical sensor⁷ or spectrometric techniques such as inductively coupled plasma-optical emission spectrometry,⁸ inductively coupled plasma mass spectrometry,⁹ flame atomic absorption spectrometry (FAAS),^{10–15} graphite furnace atomic absorption spectrometry (GFAAS).^{16–18} Several extraction and preconcentration techniques have already been proposed for Ag determination, such as solid phase extraction (SPE),^{19–21} cloud point extraction (CPE),^{10–13,22} and dispersive liquid–liquid microextraction (DLLME).^{14–18} These procedures have been applied with good results, although using relatively simple samples, such as water. Among the extraction procedures mentioned, DLLME appears as an interesting technique. The DLLME procedure is based on the introduction of a micro-volume of an organic solvent, together with a dispersing solvent in an aqueous sample containing the analyte, leading to the formation of a cloudy solution with fine droplets. Soon afterwards, this cloudy solution must be centrifuged to accelerate the sedimentation of the fine droplets formed by the extracting solvent. In this extraction method, the analyte present in solution, either in the form of a complex or not, is transferred to the droplets of the extracting solvent, consequently concentrating the extracted analyte in the small volume of the organic phase formed. Then, the determination of the analyte in the organic phase can be accomplished instrumentally.^{23–25} An immediate advantage associated to this technique is the demand for low volumes of organic solvents, therefore minimizing health hazards and environmental concerns associated to the handling and use of toxic organic solvents. Furthermore, DLLME is fast, inexpensive, highly sensitive and can be effectively used for matrix separation and, consequently, for the reduction of potential interferences in the determination.^{26–31}

The purpose of the present work was to develop a new method for Ag determination in biological samples. The step of extraction and preconcentration was performed using DLLME with ammonium *O,O*-diethyl dithiophosphate as the chelating agent and quantitative analysis was performed using graphite furnace atomic absorption spectrometry (GFAAS).

Experimental

Instrumentation

All experiments were carried out using an AAnalyst 100 (Perkin Elmer, Norwalk, CT, USA) atomic absorption spectrometer with deuterium-arc background correction, equipped with an HGA 800 graphite tube atomizer. Integrated absorbance (peak area) was used exclusively for signal evaluation. A silver hollow cathode lamp (Perkin Elmer) was operated at 10 mA and the main Ag line at 328.1 nm was used for all experiments, with a spectral slitwidth of 0.7 nm. Longitudinally heated pyrolytic graphite-coated graphite tubes with pyrolytically coated L'vov platforms were used throughout this work. L'vov platforms were thermally treated with tungsten (Merck, Darmstadt, Germany), by means of 10 injections of 40 μL each of a 1000 mg L^{-1} W solution (Merck), and applying the temperature program shown in Table 1 after each injection, resulting in a total deposited mass of 400 $\mu\text{g W}$.

Samples were digested using an Ethos Plus (Milestone, Sorisole, Italy) microwave oven. An Excelsa Baby II 206-R centrifuge (Fanem, São Paulo, Brazil) was used for the preconcentration procedure.

Reagents, standards and samples

All chemicals were at least of analytical-reagent grade. Water was deionized to a resistivity of 18.2 $\text{M}\Omega\text{ cm}$ in a Milli-Q system (Millipore, Bedford, MA, USA). Nitric acid (Merck) was doubly distilled in a sub-boiling quartz distillation apparatus (Kürner Analysentechnik, Rosenheim, Germany). Ammonium *O,O*-diethyl dithiophosphate (DDTP, Aldrich, Milwaukee, WI, USA) was used after purification in a C18 silica gel column (Fluka, Switzerland). Acetone, ethanol, *n*-propanol, chloroform, dichloromethane (Nuclear, Diadema, São Paulo, Brazil), carbon tetrachloride (Synth, Diadema, São Paulo, Brazil) and hydrogen peroxide (Suprapur Perhydrol 30%, Merck) were used without further purification. Working solutions were prepared in 0.3 mol L^{-1} HNO_3 by proper dilutions of the stock 1000 mg L^{-1} solution of Ag (Sigma-Aldrich). Argon 99.996% (White Martins, São Paulo, Brazil) was used as purge and protective gas.

Table 1 Heating program of graphite furnace for coating L'vov platform with W and graphite furnace temperature program for Ag determination in biological samples

	Step	Temperature/ $^{\circ}\text{C}$	Ramp/ $^{\circ}\text{C s}^{-1}$	Hold/s	Ar flow rate/ mL min^{-1}
Coating L'vov platform	1	120	5	25	250
	2	150	10	60	250
	3	600	20	15	250
	4	1000	10	15	0
	5	1400	10	5	250
	6	2000	3	2	250
Ag determination	Drying I	80	10	10	250
	Drying II	120	10	10	250
	Pyrolysis	1100	10	10	250
	Atomization	1800	0	5	0
	Cleanout	2000	1	5	250
	Cooling	20	1	5	250

The certified reference materials used for accuracy verification were DOLT-3 dogfish liver and DORM-2 dogfish muscle, from the National Research Council Canada (Ottawa, ON, Canada), and NIST 1577b bovine liver, from the National Institute of Standards & Technology (Gaithersburg, USA).

Sample preparation procedure

Samples were weighed directly into PFA flasks in aliquots containing approximately 50 mg or 500 mg, depending on the sample. Aliquots of 1.0 mL of HNO_3 and 0.5 mL of H_2O_2 were added to each flask containing 50 mg of sample, whereas aliquots of 4.0 mL of HNO_3 and 1.0 mL of H_2O_2 were added to each flask containing 500 mg of sample. In both cases, the resulting mixture was submitted microwave-assisted heating, to 180 °C at a rate of 16 °C min^{-1} for 10 min, followed by cooling to room temperature. The digested samples were transferred to polypropylene tubes (Sarstedt, Nümbrecht, Germany) and added with deionized water up to the final volume (50 mL). The resulting solutions were kept under refrigeration until use.

DLLME procedure

Aliquots of 5.0 mL of the digested samples were transferred to 14 mL polypropylene tubes (Sarstedt). Then, 100 μL of a 0.5% m/v DDTP solution was added, followed by a mixture of 500 μL of acetone (dispersing solvent) and 80 μL of chloroform (extracting solvent). This mixture was manually agitated for approximately 30 s, resulting in a cloudy solution, and maintained in an ultrasonic bath for 10 min. In this step, the Ag^+ ions react with DDTP and are extracted into the fine droplets of chloroform dispersed in the solution. Afterwards, the finely dispersed droplets of chloroform were aggregated in the bottom of the flask by centrifugation for 2 min at 1500 rpm, resulting in an organic phase of approximately 40 μL . An aliquot of 20 μL of the organic phase was injected manually into the graphite furnace and submitted to the temperature program shown in Table 1. Calibration curves were carried out with and without pre-concentration, as a means to evaluate the enhancement factor associated to the extraction procedure.

Results and discussion

Evaluation of pyrolysis and atomization temperatures

Pyrolysis and atomization temperatures were optimized for Ag in an aqueous standard and in DOLT-3 sample, both submitted to DLLME. The results are shown in Fig. 1. In order to avoid infiltration of the organic solvent into the graphite pores, which leads to poor atomization efficiency, the pyrolytic graphite platform was submitted to a thermal treatment with tungsten, resulting in the deposition of 400 μg W onto the platform surface. The intention in this case was to reduce the porosity of the graphite surface by means of the carbide-forming reaction of W.³¹ Under these conditions, it could be observed that the thermal behavior for Ag in the sample and in the aqueous standard were quite similar, and that Ag was stable up to a pyrolysis temperature of 1200 °C in CHCl_3 extracts obtained from DOLT-3 and 1300 °C in the same extract obtained from an

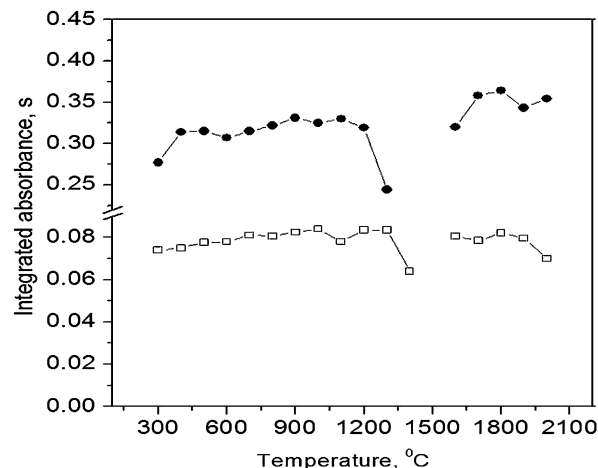


Fig. 1 Pyrolysis and atomization temperature curves for Ag in CHCl_3 extracts obtained from DLLME applied to (—●—) DOLT-3 and (—□—) 0.02 $\mu\text{g L}^{-1}$ aqueous standard. Conditions: 5.0 mL of sample; 0.01% m/v DDTP; 500 μL acetone; 80 μL chloroform.

aqueous standard. The thermal stability of Ag was remarkably improved in the organic extract when compared to an acidified aqueous solution, which may be attributed to the modifier effect of DDTP, which bonds strongly to Ag in the condensed phase. As a compromise, a pyrolysis temperature of 1100 °C was adopted for subsequent experiments. The atomization temperature was optimized in a similar way to the pyrolysis temperature; an atomization temperature of 1800 °C was finally selected.

Evaluation of DLLME extraction parameters

Effect of pH. The efficiency of complexation of analytes with most chelating agents is frequently a pH-dependent parameter. However, complex forming reactions between DDTP and most transition metals are efficiently carried out under acidic conditions, therefore eliminating the need for a thorough optimization of the pH.²⁴ Nonetheless, DDTP can be decomposed under oxidizing conditions, such as those imposed by relatively high concentrations of HNO_3 . Hence, the final HNO_3 concentration used to carry out DLLME was optimized using the DOLT-3 sample and also for an aqueous standard containing 0.02 $\mu\text{g L}^{-1}$ Ag; the results are shown in Fig. 2. In this figure, it is possible to observe that the signal intensity increases gradually up to a HNO_3 concentration of 0.3 mol L^{-1} , whereas for higher concentrations the signal decreases significantly. This fact can be attributed to DDTP degradation, as previously mentioned.²⁵ The final HNO_3 concentration used for calibration solutions and digested samples was selected as 0.3 mol L^{-1} .

Effect of extracting and dispersing solvents. The main requirements for an extracting solvent in DLLME include density higher than water and low water solubility. The efficiency of chloroform and dichloromethane as extracting solvents was evaluated, and high signal intensity was obtained using chloroform. Moreover, chloroform was preferred as the extracting solvent basically due to its cost-effectiveness, when

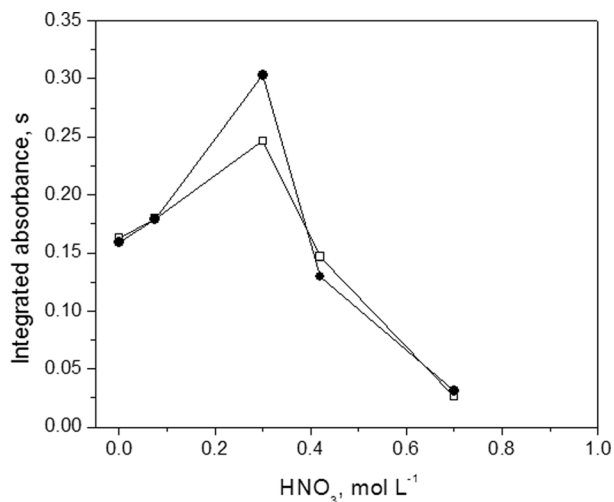


Fig. 2 Influence of HNO₃ concentration on the analytical signal for Ag obtained from DLLME applied to (—●—) DOLT-3 and a (—□—) 0.02 μg L⁻¹ aqueous standard. Conditions: pyrolysis temperature 1100 °C; atomization temperature 1800 °C; 5.0 mL sample, 0.01% m/v DDTP; 500 μL acetone; 80 μL chloroform.

compared to the other solvents. Although only about 50% of the initially added chloroform could be recovered by the end of the extraction process, the extraction efficiency was latter proven to be significantly high.

In Fig. 3(A), the influence of the volume of the extracting solvent on the analytical signal for the DOLT-3 sample and an aqueous standard submitted to DLLME is shown. The chloroform volume was varied from 50 to 200 μL. The results have shown that with the addition of 50 μL of chloroform, phase separation could not be achieved, which is due to complete solubilization of the organic solvent. The highest signal was obtained by the addition of 80 μL of chloroform, which resulted in an organic phase with approximately 40 μL. The adoption of larger volumes resulted in gradual decrease of the analytical signal, which occurs as a consequence of higher dilution of the analyte in the organic phase. Any potential improvement in the extraction efficiency that may result from the use of larger volumes of the extracting solvent is, therefore, overcome by a reduction in the preconcentration factor, so a chloroform volume of 80 μL was adopted for further experiments.

In DLLME, the selection of the dispersing solvent is also an important factor. This solvent should be able to solubilize the extracting solvent and should also be soluble in water in order to facilitate the formation of microdroplets of the extracting solvent and, consequently, the transfer of the complex formed in the aqueous phase to the organic phase. In order to cope with these characteristics, acetone was used as dispersing solvent and an optimization of its volume was performed. The results are shown in Fig. 3(B).

The volume of the dispersing solvent should be the as small as possible to allow an efficient dispersion and recovery of the extracting solvent. The maximum signal intensity was obtained using 500 μL of acetone, for both sample and aqueous solution. The low sensitivity observed with the use of volumes inferior to

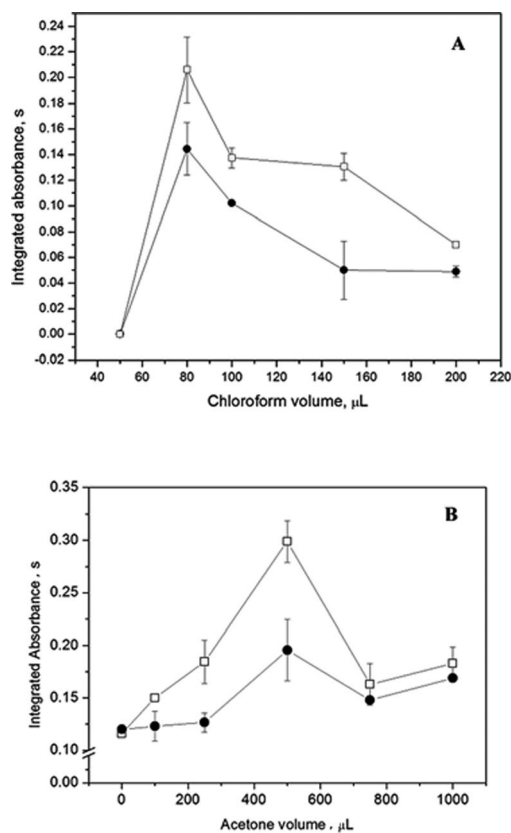


Fig. 3 (A) Effect of chloroform volume used for pre-concentration of Ag by DLLME using 500 μL acetone as dispersing solvent and (B) influence of acetone volume on the pre-concentration of Ag by DLLME using 80 μL chloroform as extractant. Legend: (—●—) DOLT-3 sample and (—□—) aqueous solution containing 0.02 μg L⁻¹ Ag. Conditions: pyrolysis temperature 1100 °C; atomization temperature 1800 °C; 5.0 mL of sample, 0.01% m/v DDTP.

200 μL can be ascribed to less efficient dispersion, resulting in a reduction in the surface area of the extracting solvent due to the formation of larger droplets and, consequently, inferior extraction efficiency. Adoption of volumes larger than 600 μL leads to a decrease in the analytical signal, despite the potentially improved dispersion and mass transfer. This is believed to be due to an increase in the volume of the organic phase, resulting in lower preconcentration factor due to dilution of the analyte in the organic phase. The use of 500 μL acetone was adopted for further experiments, allowing the recovery of 40 μL of the organic phase, which was sufficient to perform two measurements of a single sample aliquot.

Effect of DDTP concentration and extraction time. The choice of DDTP as the chelating agent was based on its characteristics, which include reasonable solubility in water and high stability in acidic medium. The variation of the signal intensity as a function of the concentration of DDTP for the sample and aqueous solution submitted to DLLME is shown in Fig. 4(A). It can be observed that the signal reaches a maximum for a concentration of 0.01% m/v of the chelating agent, both in the DOLT-3 sample and in an aqueous solution. The sensitivity obtained using a DDTP concentration lower than 0.01% m/v is

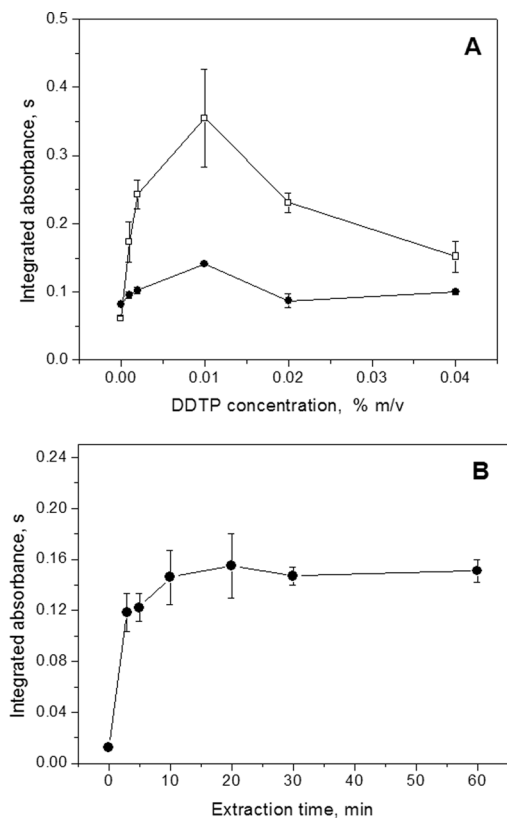


Fig. 4 (A) Influence of DDTP concentration on the pre-concentration of Ag by DLLME and GFAAS and (B) influence of the extraction time on the pre-concentration of Ag by DLLME and GFAAS using 0.01% m/v DDTP. Legend: (-●-) DOLT-3 sample and in (-□-) aqueous solution 0.02 $\mu\text{g L}^{-1}$ Ag. Conditions: pyrolysis temperature 1100 °C; atomization temperature 1800 °C; 5.0 mL of sample; 500 μL acetone; 80 μL chloroform.

significantly reduced, particularly in the aqueous solution, which is most likely due to the low extraction efficiency as a consequence of incomplete complexation.

It is interesting to notice that for the DOLT-3 sample a significant fraction of the analyte can be extracted even without the use of DDTP. This effect may be attributed to the presence of concomitant ions in the sample solution originating from the sample, generating hydrophobic complexes or ion pairs that may be co-extracted to the organic phase.^{32–35} Nevertheless, in this sample, an improvement of approximately 40% in sensitivity is achieved using DDTP at 0.01% m/v, justifying its use. DDTP concentrations above 0.01% m/v cause a decrease in the analytical signal, which may be due to saturation of the organic droplets or formation of charged complexes, with a consequent decrease in the extraction efficiency.

The extraction time is an important factor in procedures involving liquid–liquid extraction, as it reflects the efficiency of the mass transfer process of the analyte from the aqueous phase to the organic phase. The extraction time was defined as the interval between the addition of the extracting solvent and the centrifugation step. The influence of the extraction time for Ag in the sample DOLT-3 is shown in Fig. 4(B). Considering the

Table 2 Figures of merit obtained for the determination of Ag in biological samples using DLLME and GFAAS

Parameter	Values
Working range ($\mu\text{g L}^{-1}$)	0.008–0.056
Slope ($\text{s L } \mu\text{g}^{-1}$)	0.00328
R	0.9997
LOD (ng g^{-1})	2
RSD (%)	4–13
Enhancement factor (EF)	70

Table 3 Results obtained for the determination of Ag in biological samples following DLLME and GFAAS

Sample	Certified ($\mu\text{g g}^{-1}$)	Found ($\mu\text{g g}^{-1}$)
DOLT-3 (dogfish liver)	1.20 \pm 0.07	1.27 \pm 0.18
DORM-2 (dogfish muscle)	0.041 \pm 0.013	0.037 \pm 0.004
NIST 1577b (bovine liver)	0.039 \pm 0.007	0.035 \pm 0.012

relative standard deviation of the measurements, the equilibrium is reached quite quickly, in approximately 5 min. Nevertheless, in order to assure maximum extraction and, consequently, higher sensitivity, a 10 min extraction period was adopted for further experiments.

Figures of merit

The figures of merit obtained for the determination of Ag in biological samples using DLLME and GFAAS are presented in Table 2. The working range employed for the determination of Ag was in the order of ng L^{-1} . A linear correlation coefficient higher than 0.999 was obtained for the calibration curve using aqueous standards submitted to the DLLME procedure. The enhancement factor, represented by the ratio between the slopes of calibration curves obtained with and without pre-concentration, was determined as 70. The detection limit (LOD) was calculated as the ratio between 3 times the standard deviation of ten blank readings and the slope of the calibration curve submitted to the pre-concentration procedure, also taking into account the necessary dilutions. The determined LOD obtained using DLLME is in the order of ng g^{-1} , demonstrating that association of DLLME with GFAAS is characterized by a remarkably high detection capability, which is frequently necessary for the determination of Ag in biological samples. The precision, measured as the RSD, was typically between 4 and 13%, which is adequate, considering that manual pipetting of the organic phase has been adopted when using the DLLME procedure.

Analytical application

The procedure was applied for the determination of Ag in digested biological samples. Three certified reference materials were analyzed, and the results are shown in Table 3. All the determined values are in good agreement with the certified values at a 95% statistical confidence level, indicating good accuracy of the proposed procedure.

Conclusions

Dispersive liquid–liquid microextraction using GFAAS has proved to be a simple, fast, and efficient technique for the determination of Ag in biological samples. Good detection limit, accuracy and enhancement factor were obtained for a virtually interference-free procedure, which is at least comparable to other preconcentration methods reported for Ag determination. The method may probably be extended to other samples with complex matrices and analytes that form complexes with DDTP.

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