Technical note

Cadmium determination in biological samples by direct solid sampling flame atomic absorption spectrometry

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Abstract

A direct solid sampling flame atomic absorption spectrometric procedure for trace determination of cadmium in biological samples has been developed. Test samples (0.05–2.00 mg) were ground and weighed into small polyethylene vials, which were connected to the device for solid sample introduction into a conventional air/acetylene flame. Test samples were carried as a dry aerosol to a quartz cell, placed between the burner and the optical path, which had a perpendicular entrance and a slit in the upper part. The atomic vapor generated in the flame produced a transient signal that was totally integrated within 1 s. The effect of operating conditions and the extent of grinding on the analytical signal were evaluated. Background signals were always low and a characteristic mass of 0.29 ng Cd was obtained. Calibration was performed using different masses of solid certified reference materials. Results obtained for certified and in-house reference materials were typically within the 95% confidence interval of the certified and/or reference value, and the precision, expressed as relative standard deviation, was between 3.8 and 6.7%. The proposed system is simple and it might be adapted to conventional atomic absorption spectrometers allowing the determination of Cd in more than 80 test samples per hour, excluding weighing.

Keywords: Direct solid analysis; Flame atomic absorption spectrometry; Cadmium determination; Biological samples

1. Introduction

Different procedures have been developed for atomic absorption analysis of solid samples and they have received considerably increasing attention during the last years. Direct solid analysis (DSA) has been referred as a powerful technique in view of its detection limits, achievable for a variety of elements [1]. Since the beginning of atomic absorption technique, many authors have been developing devices for DSA, which presented advantages, particularly for samples that are difficult to handle by conventional sample digestion procedures [2–5].
Nowadays graphite tube atomizers are most frequently used for DSA because of their higher sensitivity and the ease of sample introduction compared to procedures with flame atomizers [6]. Inductively coupled plasma optical emission spectrometry with previous vaporization of the sample has also been used for DSA [7,8]. In graphite furnace atomic absorption spectrometry (GF AAS), solid samples can either be introducing into the furnace directly [6,9], or indirectly as a slurry [10,11], which allows the use of only slightly modified instrumentation [11–13]. However, particle size must be carefully controlled, as it affects slurry stability and atomization efficiency [11]. In general, slurry sampling and DSA techniques using graphite furnace atomizers provide several advantages including reduced sample preparation time, higher relative sensitivity and decreased risk of loss (or contamination) of analyte during sample pretreatment [1,6].

In spite of a few moderately successful approaches in the past, direct introduction of solids into flames is difficult [6,14–18]. The introduction of solid samples as slurries appears to be easier [19], but problems, such as the blockage of the nebulizer were reported when slurries were aspirated directly [20,21]. A separation of the vaporization and the atomization process was proposed in order to overcome these problems [22–24].

The introduction of small pieces of filter paper containing the test sample inside a graphite tube into an air/acetylene flame was proposed as an alternative for the determination of lead in sediments [25]. More recently, a simple device for the determination of copper in bovine liver by flame-DSA was introduced [26]. In this approach, ground solid samples were carried with a gas stream as a dry aerosol through a heated quartz cell directly into an air/acetylene flame. Good accuracy and relatively low detection limits (8 ng, absolute) were obtained with the proposed device.

In this work a device similar to the previously described one [26] was investigated for cadmium determination in biological samples by flame-DSA. Different biological reference materials were investigated, and results were compared with those obtained with a conventional sample digestion procedure. The effect of particle size and operating conditions on the integrated absorbance signals was also investigated.

2. Experimental

2.1. Instrumentation

All flame measurements were carried out using a Model Vario 6 FL atomic absorption spectrometer (Analytik Jena AG, Germany), equipped with a deuterium background corrector and a hollow-cathode lamp for cadmium, operated at 5 mA (wavelength 228.8 nm, spectral bandwidth 0.5 nm). A conventional air/acetylene burner (10 cm slit) was used throughout. Integrated absorbance with an integration time of 1 s was used for signal evaluation. An Analytik Jena Model AAS5 EA atomic absorption spectrometer, equipped with a Model MPE 5 autosampler was used for measurements by GF AAS. Optical parameters for Cd determination were the same described above.

A microwave oven with an exhaust unit (nominal maximum power of 1000 W, Provecto, DGT-100, Campinas, Brazil), with six polytetrafluorethylene (PTFE) high-pressure digestion vessels (90 ml), was used for comparative studies based on sample digestion with nitric acid–hydrogen peroxide.

A microbalance Model M2P (Sartorius, Göttingen, Germany) with a resolution of 1 μg and an electronic weighing range up to 2 g was used for weighing the test samples.

2.2. Flame-DSA system for Cd determination

A schematic diagram of the device used in this work is shown in Fig. 1. More details were
presented in Ref. [26]. A glass sampling chamber (S) assembled to a glass manifold was fitted to two valves (V1 and V2) and a flow meter (F) to control the air flow in the system. A column filled with silica was used to remove the moisture of the air. The glass tubes were connected to a T-quartz cell (8 mm inner diameter and 40 mm length with a slit of 2 mm), which was positioned 4–8 mm above the burner in an air–acetylene flame. Test samples (0.05–2.00 mg) were weighed into polyethylene (PE) vials. When valve V2 was closed the vials could be fitted to the sampling chamber (S). After the valve V2 was opened the main stream of air was changed, passing through the PE vial, and the test sample was carried to quartz cell. When air flows through the sampling chamber the test sample is blown out to the quartz cell and burnt in the flame. The optical beam passes approximately 10 mm above the quartz cell. The total air flow-rate through the quartz cell is kept constant at 5 l min⁻¹ in all stages. Transient signals were completely recorded within 1 s.

2.3. Samples and reagents

The following biological reference materials were used in this work: BCR 186 pig kidney (Community Bureau of Reference, Brussels, Belgium), NIST SRM 1566a oyster tissue (National Institute of Standards and Technology, Gaithersburg, MD), DOLT-2 dogfish muscle and liver and TORT-2 lobster hepatopancreas (National Research Council Canada, Ottawa, Ont., Canada). Three in-house fish tissue samples were lyophilized and ground in an agate mortar. Cadmium was determined in these materials using digestion according to the procedure described in Section 2.4 and GF AAS conditions according to Ref. [27], to confirm the established values. As there was no significant difference between the determined and the previously established values, the latter ones were used as reference for subsequent studies. Cadmium determination by GF AAS as also performed in river fish tissue samples and results varied from 2.08 ± 0.13 to 11.8 ± 0.6 μg g⁻¹. These results were compared with those using the proposed procedure by flame-DSA. Lyophilized samples were dried at 70 °C for 4 h and kept in a desiccator prior to the Cd determination. For the conventional digestion, reagents were of Suprapur® or analytical grade (Merck, Darmstadt, Germany), and nitric acid was doubly distilled in a subboiling system (Berghof, model BSP 929, Germany). All samples were classified in different particle size fractions: ≤ 30 and ≤ 50 and ≤ 80 μm using polyester sieves.

2.4. Sample digestion

Samples (~0.2 g) were weighed, and 2.5 ml of concentrated nitric acid and 2 ml of 30% hydrogen peroxide were added. After 1 h for initial acid attack, the PTFE vessels were capped and heated using the following program in the microwave oven: 6 min at 400 W, 5 min at 0 W, 1 min at 630 W, 5 min at 400 W, 5 min at 0 W and 5 min at 400 W. Finally, digests were diluted with water to 25 ml and analyzed by GF AAS.

3. Results and discussion

3.1. Optimization of operating conditions for the flame-DSA procedure

Initial experiments were performed using DOLT-2 reference material to evaluate the influence of flame composition on integrated absorbance signals with the proposed flame-DSA procedure. The behavior of other reference materials was also investigated, but no differences were observed. Integrated absorbance signals were converted to characteristic mass to facilitate comparison. For the optimization studies the distance between the quartz cell and the burner was kept at 5 mm, and the particle size of test sample was ≤ 80 μm.

The influence of flame composition on signals for Cd is shown in Fig. 2. The acetylene flow rate was kept constant at 2 l min⁻¹ and the air flow rate was varied from 8 to 12 l min⁻¹, and best results (lowest characteristic mass) were obtained for air flow rates of 11–12 l min⁻¹. The mixture of 11 l min⁻¹ air + 2 l min⁻¹ acetylene was chosen for further experiments due to its high sensitivity and the uniform and reproducible peak shapes.
These results are similar to those reported in Ref. [26] for copper, using a similar device for flame-DSA. Possible memory effects were evaluated by further injection of air in the system with the PE vial empty. For all measurements memory effects were insignificant, and the relative standard deviation was better than 6%. Background signals were always smaller than 0.05 A in peak height for all investigated air flow rates.

Fig. 3 shows the influence of the distance between the quartz cell and the optical path from 6 to 10 mm on the integrated absorbance signal for Cd. A decrease of the characteristic mass from 0.39 to 0.29 ng Cd was observed with increasing distance. Ten millimeter was the maximum distance tested due to mechanical limitations of the equipment. The results are in agreement with those described in previous work [24], where a characteristic mass of approximately 0.2 ng Cd was found, using different devices for sample pre-treatment and atomization.

### 3.2. Influence of particle size on signals by flame-DSA

An interesting feature of the proposed system is the previous heating (maybe combustion) of the sample inside the quartz cell before it enters the flame. Another aspect of the proposed system is that the test samples enter into the flame as a dry aerosol and the loss of heat is minimal. A bright light emission was observed when the sample

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**Fig. 2.** Influence of flame composition (acetylene flow of 2 l min$^{-1}$) on integrated absorbance for Cd in DOLT-2 reference material. Air flow in the sampling chamber: 5 l min$^{-1}$; vertical lines represent the relative standard deviation ($n=6$).

**Fig. 3.** Influence of the distance between the quartz cell and the optical path on the integrated absorbance for Cd; vertical lines represent the relative standard deviation ($n=5$).
exited from the quartz cell and started to burn in the flame. Fig. 4 shows a typical signal recorded for Cd using the proposed flame-DSA system and the small background signal.

In order to evaluate the influence of particle size, the samples were classified into three size fractions: \(<30\), \(<50\), and \(<80\) \(\mu\)m. It could be expected that samples with smaller particle size might improve the signals for Cd. However, no such signal improvement was observed with the three particle size fractions studied. This fact was elucidated by the determination of the relative number of particles in sub-fractions ranging from 10 mm to 10 mm for the three selected fractions. Between 1400 and 2700 particles were counted for each fraction and the results showed that from 92 to 97\% of the particles were below 10 \(\mu\)m for the \(<80\) \(\mu\)m’ fractions, and more than 96\% of the particles were \(<20\) \(\mu\)m. For in-house samples of fish tissue 91–96.5\%, more than 94\% and more than 97\% of the particles were below 10 \(\mu\)m in the \(<80\), \(<50\), and \(<30\) \(\mu\)m fraction, respectively. These results show that most of the particles are very small, explaining the apparent non-dependence of the Cd signal on particle. Therefore, particle sizes \(<80\) \(\mu\)m were used in the proposed system for subsequent studies.

3.3. Calibration

Quite often, different masses of the same reference material are used for calibration in DSA–AAS, and a limitation of this procedure is that reference materials of a composition similar to the samples have to be used. In this procedure a good correlation between different masses of Cd from different reference materials was observed as can be seen in Fig. 5. With 25 points (minimum of 4 points for each test sample) the analytical curve shows a correlation of 0.9983 \((R)\). The cadmium concentration in fish tissue samples and the reference materials was determined using this correlation curve for calibration. All results, except that for TORT-2, were within the 95\% confidence intervals of the certificate and/or of the comparative values using acid digestion and GF AAS, as shown in Table 1. The relative standard deviation was between 3.8 and 6.7\% \((n=5)\).

3.4. Figures of merit

Table 2 shows the analytical figures of merit for the proposed flame-DSA method. The characteristic mass of 0.29 ng/0.0044 s was close to the values reported previously [24], using a vaporization device before atomization by flame AAS. The sensitivity was suitable for routine determination of Cd in biological matrices, and the absolute limit of detection (LOD) was 0.27 ng \((3\ s)\) or 0.27 \(\mu\)g \(\text{g}^{-1}\) if a sample mass of 1 mg is used. These values are better when compared to conventional flame AAS, where LOD values approximately 10 \(\mu\)g \(\text{g}^{-1}\) are obtained, assuming the digestion of 0.1 g of sample and a dilution to 25 ml.
Fig. 5. Correlation between integrated absorbance and mass of Cd in certified reference materials using the proposed flame-DSA procedure ($y = 0.0148x + 0.0066; R^2 = 0.9967$).

Excluding the weighing step it is possible to perform approximately 80 determinations per hour, using the proposed procedure. However, it is necessary to keep the weighed samples in a dry environment to avoid problems related to particle adsorption on the internal surfaces of the system. The use of solid reference materials for calibration may be a limitation for materials, for which no reference materials are available. However, the possibility of coupling to an inexpensive flame atomic absorption spectrometer, and a short time needed for the individual determinations, makes this procedure effective and, like others simple devices [28], an alternative to conventional techniques.

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References


