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Analytical Methods Section

Slurry atomization of vegetables for the electrothermal atomic absorption spectrometric analysis of lead and cadmium

P. Viñas, N. Campillo, I. López García & M. Hernández Córdoba*

Department of Analytical Chemistry, Faculty of Chemistry, University of Murcia, E-30071-Murcia, Spain

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Slurry-ETAAS procedures for the rapid determination of lead and cadmium in vegetables are described. Fast-programme methodology, in which the drying and ashing stages are replaced by a single modified drying step, is used. Slurries are prepared in a 20% (v/v) ethanol medium using 0·1% ammonium dihydrogenphosphate as a chemical modifier. The suspending medium also contains hydrogen peroxide to prevent the build-up of carbonaceous residue inside the atomizer. Platform atomization using 1800°C and 1700°C for lead and cadmium, respectively, permits calibration with aqueous standards. Results for two standard reference materials confirm the reliability of the procedures.

INTRODUCTION

The conventional way to determine trace metals in foods by means of electrothermal atomic absorption spectrometry (ETAAS) requires a prior dissolution stage of the solid samples. This can be problematic when dealing with very low levels of determinand since the prolonged sample manipulation can lead to contamination or determinand loss, and there are, in practice, severe problems of reproducibility. One way to overcome the problem and thus obtain reliable results with improved reproducibility is to use the slurry approach in which, instead of a solution, a suspension prepared from the finely powdered sample is introduced into the atomizer. Analysts with no previous experience of this methodology might doubt the reliability of the results thus obtained. However, a number of well-founded papers published during the last years and summarized recently (Bendicho & de Loos-Vollebregt, 1991), have proved that, when appropriate experimental conditions are used, the slurry approach provides satisfactory results with a saving of both time and reagents. In the case of lead and cadmium determination in vegetables, several successful procedures have been described (Stephen *et al.*, 1985, 1987; Fagioli *et al.*, 1986; Hoenig & Van Hoeyweghen, 1986; Olayinka *et al.*, 1986; Ebdon & Lechotycky, 1986, 1987; Carrión *et al.*, 1988; Miller-Ihli, 1988, 1993; Lynch & Littlejohn, 1989, 1990; Ebdon *et al.*, 1990; Yu *et al.*, 1990; Hernández Córdoba & López Garcia, 1991; Dolinsek *et al.*, 1991).

* To whom correspondence should be addressed.

On the other hand, as Halls showed (Halls, 1984) in many instances, conventional heating programmes can be simplified by replacing the drying and ashing steps by a modified drying stage. This so-called fast-programme methodology considerably shortens the heating programme.

This paper describes a study of the determination of lead and cadmium in vegetables with both slurry and fast-programme methodologies. The use of platform atomization in the presence of phosphate as a matrix modifier allows very simple calibration with aqueous standards. It is interesting to note that the addition of hydrogen peroxide to the suspension medium greatly decreases the build-up of carbonaceous residues inside the atomizer. The procedures and data here reported using an unmodified commercial atomizer and low-cost reagents can be of practical use to those interested in determining very low amounts of lead and cadmium in plant materials.

MATERIALS AND METHODS

Apparatus and reagents

A Perkin-Elmer Model 1100B atomic absorption spectrometer equipped with deuterium-arc background correction and an HGA-400 (Perkin-Elmer) graphite furnace atomizer were used. Pyrolytic graphite platforms inserted into pyrolytic graphite coated tubes were obtained from Perkin-Elmer (Part B013-5653). Measurements were performed at 283·3 and 228·8 nm using conventional hollow cathode lamps (Perkin-Elmer)

and bandwidths of 0·7 nm and 0·7 nm for lead and cadmium, respectively. Argon was used as the inert gas at 300 ml min^{-1}, except during the atomization step, where the flow was stopped. Background-corrected integrated absorbance was used as the analytical signal. Optimization of the temperature for the drying stage was performed experimentally by using a thermocouple, together with a personal computer as detailed elsewhere (López García *et al.*, 1993). Homogenization of the suspensions was carried out by using a T25 Ultra-Turrax homogenizer. A Branson ultrasound bath was used to sonicate the slurries. Particle size was estimated by using an Imco 10 Image Computer (Kontron).

Severe problems of contamination arising from the micropipette tips, plasticware and, to a lesser extent, glassware were found, especially when determining cadmium. To avoid this, all the volumetric material used was immersed overnight in a solution containing 10% hydrochloric and 20% nitric acids and thoroughly washed with pure water immediately before use. When this precaution was followed, plastic vessels of the type commonly used to collect clinical samples were found suitable for the slurry preparation. Redistilled water obtained in our laboratory proved inadequate and high-quality water obtained using a Milli-Q system (Millipore) was used exclusively. Stock aqueous standards of cadmium and lead (1000 μg ml^{-1}) were obtained from Panreac (Spain). Working standards were prepared daily by appropriate dilution. Other chemicals were obtained from Fluka and were used as received. The data reported here were obtained in a laboratory with no special precautions against dust or atmospheric contamination, but the use of a clean-room, if available, is recommended.

Procedures

Most of the samples were collected from near Murcia (Spain), and those of frozen peas and spinach were purchased from a supermarket. The samples were thoroughly washed with water, chopped, dried in an oven at 90°C to constant weight and then ground in a domestic grinder for about 2 min. They were maintained in tightly closed plastic vessels until the suspensions were prepared.

Slurries were prepared by weighing amounts of sample ranging from 10 to 500 mg in plastic vessels and by adding ethanol (5 ml), concentrated (33% w/v) hydrogen peroxide (2 ml) and ammonium dihydrogenphosphate (25 mg), making up to a final volume of 25 ml with water. Then the suspensions were homogenized for 10 min and sonicated for another 5 min. Next, while the suspensions were being continuously stirred by means of a magnetic stirrer, 20 μl aliquots were taken and placed in the atomizer. The optimized heating programmes are given in Table 1. Calibration was performed by using aqueous standards under identical experimental conditions. Appropriate blanks containing all the reagents but no determinand were used to correct the results.

Table 1. Fast-furnace programmes for the determination of lead and cadmium using platform atomization

Element	Step	Temperature (°C)	Ramp (s)	Hold (s)
Lead	Dry	230	5	35
	Atomize[a]	1 800	0	3
	Clean	2 650	1	3
Cadmium	Dry	400	10	25
	Atomize[a]	1 700	0	3
	Clean	2 650	1	3

[a] The gas flow was stopped.

RESULTS AND DISCUSSION

For the slurry-ETAAS approach to be successful, a low particle size is important. As has been proved by Miller-Ihli (Miller-Ihli, 1988, 1993), this condition is not so critical when an autosampler equipped with an ultrasonic mixing accessory is available and, especially, when dealing with slurries prepared from biological materials, because these samples are easily ashed inside the atomizer. However, even for this type of sample, a low particle size is desirable when using a manual micropipette, if representative and reproducible sampling is to be obtained. To check this point, a number of slurries were prepared from the vegetable samples, ground in a domestic grinder and submitted to different homogenization times. Aliquots were taken and the analytical signal from lead obtained. In this way, it was shown that a homogenization time of 10 min was necessary to obtain maximum and reproducible signals. Using the conditions recommended for the slurry preparation, an estimation of the particle size distribution was carried out by using a computerized image analyser and the results showed that about 90% of the particles were below 30 μm.

A considerable number of experiments were devoted to studying the possible stabilization of the suspensions by adding different chemicals. For this purpose 0·4% slurries were prepared from grapefruit leaves in the presence of variable amounts of each chemical tested, the suspensions being sonicated for 5 min and then magnetically stirred for another 10 min. While the suspensions were being stirred, aliquots were taken and the lead signal obtained. The stirring was stopped, and after a 15 min interval, aliquots in duplicate were obtained from the upper portion of the supernatant and the signal from lead again obtained. Comparison of both signals permitted an estimation of the stabilizing action of the chemical. Poor or no stabilization and high background values were observed when using Triton X-100, polyvinylalcohol, polyoxyethylene sorbitan monolaurate, sorbitan monolaurate and sodium dodecylsulphate. Stabilization was better when using glycerol, but the background was very high when fast-programme heating methodology was tried. The addition of a diluted solution of ammonia was also assayed, producing a partial stabilization, but was discarded

Table 2. Extraction of lead and cadmium from vegetable slurries by addition of nitric acid

Vegetable	pH	Lead		Cadmium	
		Percent extraction	Percent in solid residue	Percent extraction	Percent in solid residue
Bean leaves	4·7	6·2	88·5	8·3	93·3
	2·9	7·2	89·7	21·7	73·3
	2·2	24·5	77·4	48·3	56·7
	1·7	30·3	66·7	66·7	36·4
	1·0	87·0	16·0	67·2	31·2
Peas	4·4	21·9	73·2	19·2	73·1
	2·7	25·0	72·6	46·1	50·0
	2·1	27·7	75·3	53·8	30·8
	1·6	48·7	51·0	64·0	34·6
	1·1	81·4	20·0	86·3	19·5

because of the build-up of carbonaceous residues inside the atomizer.

When the addition of diluted nitric acid solution to the suspensions was assayed, the signals from the supernatant were about 80% of those obtained when the suspension was sampled while being continuously stirred. This is not a stabilizing effect but a consequence of a partial extraction of the determinand to the liquid phase of the suspension. This extraction effect is beneficial for the analytical method because any increase in the degree of extraction leads to an increase in reproducibility since the errors associated with slurry sampling are minimized.

The fraction of determinand extracted into the suspending solution was studied by using a 100 ml slurry prepared from bean (*Vicia faba*) leaves. Following the addition of different amounts of nitric acid, 5 ml aliquots were taken and centrifuged. The lead and cadmium contents were then determined in the supernatant. The solid residues were washed and resuspended and their lead and cadmium contents also measured. Table 2 summarizes the results, showing the percent of determinand extracted into the liquid phase as well as the percent found in the solid residue, together with the acidity of the suspension media. As expected, the extraction efficiency increased with the concentration of nitric acid. Similar results, also summarized in Table 2, were found when the experiments were repeated using a slurry prepared from peas. However, although nitric acid helps to improve reproducibility, its use is not recommended here because the atomization of a number of suspensions containing nitric acid damages the pyrolytic material. For this reason, slurries were prepared using 25% ethanol, and aliquots were taken while the suspension was being stirred to avoid agglomeration of the particles. In this way, adequate reproducibility was obtained and the useful lifetime of the expensive pyrolytic material considerably prolonged.

Optimization of the furnace programmes

Rapid furnace programmes (Halls, 1984) are simpler than the conventional programmes as they are based on the replacement of the drying and ashing steps by a modified drying stage. The programmes were optimized for platform atomization following this methodology.

On the other hand, when atomizing suspensions prepared from biological materials, a serious problem arises in the build-up of carbonaceous residues inside the atomizer, as a consequence of incomplete ashing of the organic matrix. A way to avoid this is to include an air-ashing stage in the furnace programme (Stephen *et al.*, 1985; Ebdon *et al.*, 1990). There is no doubt that this is a well-proved solution to the problem but, unfortunately, oxygen can damage the pyrolytic material and the use of an alternative gas is not possible in several models of commercial atomizer. Recently it has been reported (Viñas *et al.*, 1994) that the addition of hydrogen peroxide to the suspension medium can greatly alleviate the problem. Apparently, the decomposition of this chemical during the heating programme plays a similar role to the use of an air-ashing stage and the build-up of residues inside the atomizer decreases. This simple approach of adding hydrogen peroxide to the slurries, as indicated in the procedure, was adopted. In all cases the suspending medium also contained 0·1% ammonium dihydrogenphosphate to avoid losses of determinand due to premature volatilization.

The temperature and holding time used in the modified drying stage were optimized first by noting sputtering signs and visual inspection of the inside of the atomizer with the aid of a dental mirror and later by using a thermocouple as indicated elsewhere (López García *et al.*, 1993). For lead determination, a 1% slurry prepared from bean leaves in the presence of 4, 8 and 16% (v/v) concentrated hydrogen peroxide was used. It was not possible to use a fast heating rate due to sputtering. The relationship between the maximum drying temperature and the minimum holding time when using a 5 s ramp is shown in Fig. 1, where data for aqueous lead standards are given for comparison.

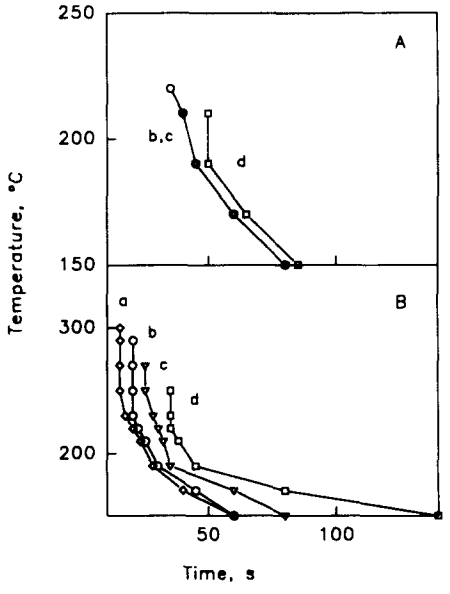

Fig. 1. Relationship between the drying temperature and drying time. A: 1% slurries containing 0·1% dihydrogenphosphate; B: aqueous lead. Hydrogen peroxide (%): (a) 0, (b) 4, (c) 8, (d) 16.

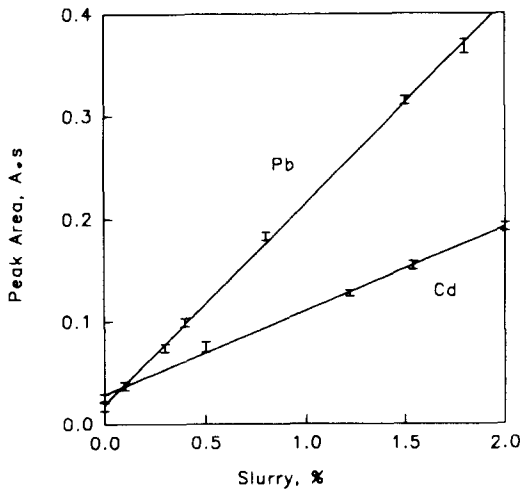

Fig. 2. Influence of slurry percentage.

As was to be expected, the higher the hydrogen peroxide concentration, the lower the maximum useful temperature that could be used to avoid sputtering. Consequently, holding time increased. A drying temperature of 230°C and a drying time of 35 s were selected. The atomization temperature was varied from 1700 to 2200°C and a maximum signal was achieved at 1800°C. A cleaning stage at 2650°C was also included in the programme.

The furnace programme for cadmium was optimized in a similar way. In this case, to decrease background, it was necessary to increase the drying temperature up to 400°C using a 10 s ramp time to avoid sputtering. Table 1 shows the furnace programmes optimized for both analytes.

Influence of slurry concentration

The results obtained when varying the slurry concentration for both cadmium and lead are shown in Fig. 2. High relative standard deviations (RSD) were found for diluted slurries. Optimal slurry percentages ranged from 0·5 to 2%, providing integrated absorbance values within the linear response range with a convenient RSD value.

Calibration and results

To check whether calibration using aqueous standards was valid, a number of suspensions were prepared from grapefruit leaves and standard additions graphs for lead and cadmium obtained. The results, which are summarized in Table 3, indicated that the gradients of these graphs were, in practice, very similar to those obtained for aqueous standards, proving the absence of a matrix effect and validating the simplest calibration with aqueous solutions. The same conclusion was reached when the experiments were repeated using slurries prepared from another two vegetable samples.

To check the reliability of the results obtained using the slurry approach here discussed, the samples were analysed for their lead content using another two different procedures. First, the samples were acid-decomposed and their lead content obtained by ETAAS following the conventional methodology. In addition to this, the lead contents were also obtained following a slurry procedure based on a partial calcination of the samples. Thus, to facilitate sample comminution and to decrease the content of organic matter, the samples were sub-mitted to a mild calcination at 500°C for 1·5 h in a muffle furnace; then, the carbonaceous residues were ground in a ball mill and the suspensions prepared using 8% glycerol instead of ethanol to obtain better stabilization. There was agreement between the different analytical approaches as deduced from the least-squares fitting of data summarized in Table 4.

Table 3. Gradients of the standard additions calibration graphs[a]

Lead		Cadmium	
Percent slurry	Gradient (A.s. ng^{-1})	Percent slurry	Gradient (A.s. pg^{-1})
0·0	0·334 4	0·00	0·004 4
0·4	0·357 0	0·15	0·004 6
0·8	0·344 3	1·00	0·004 5
1·2	0·351 6	1·60	0·004 5
1·6	0·306 3	2·00	0·004 3

[a] The gradients were calculated from four-point standard additions. Each addition point was measured three times.

In addition, vegetables were analysed to determine the cadmium content and very low values ranging from 0·01 to 0·1 $\mu g\ g^{-1}$ were found. To establish the reliability of these data, several attempts were made to obtain the cadmium content by using the two alternative procedures quoted above. However, the contamination due to sample manipulation and the high blank values made it impossible to obtain reliable values for comparison purposes as the RSD values were very high, in some cases exceeding 50%. This proves one of the advantages of the slurry procedure discussed here, as the RSD values obtained when using this approach

Table 4. Results for the determination of lead in vegetables

Vegetable	Lead ($\mu g\ g^{-1}$) (mean ± SD)		
	A	B	C
Grapefruit leaves	1·58 ± 0·01	1·48 ± 0·10	1·50 ± 0·04
Cauliflower leaves	0·68 ± 0·06	0·67 ± 0·06	0·66 ± 0·03
Spinachs	0·35 ± 0·02	0·35 ± 0·03	0·36 ± 0·13
Lyophilized peas	0·13 ± 0·02	0·12 ± 0·03	0·11 ± 0·01
Peas	0·18 ± 0·01	0·17 ± 0·01	0·17 ± 0·01
Frozen peas	0·10 ± 0·01	0·11 ± 0·02	0·12 ± 0·01
Potatoes	0·14 ± 0·01	0·14 ± 0·01	0·14 ± 0·01
Celery	0·28 ± 0·02	0·28 ± 0·02	0·27 ± 0·02
Carrots	0·33 ± 0·04	0·32 ± 0·05	0·33 ± 0·03
Bean leaves	1·13 ± 0·09	1·10 ± 0·04	1·07 ± 0·09
Citrus leaves	13·41 ± 0·97	13·18 ± 1·45	13·3 ± 2·4[a]
Apple leaves	0·48 ± 0·01	0·48 ± 0·03	0·47 ± 0·02[a]

A: Slurry procedure with calcination.
B: Slurry procedure without calcination.
C: Dry-ashing and acid dissolution procedure.
[a] Certified values.

Table 5. Cadmium content in reference materials

Sample	Cadmium, $\mu g\ g^{-1}$ (mean ± SD)	
	Slurry procedure	Certified value
Citrus leaves (SRM 1572)	0·029 ± 0·006	0·03 ± 0·01
Apple leaves (SRM 1515)	0·016 ± 0·003	0·014[a]

[a] Not certified. Value is given by the supplier for informative purposes.

were in the 5–20% range. For that reason, the reliability of the procedure studied was checked by analysing two standard reference materials. As can be seen in Table 5, there was agreement between the data obtained using the slurry procedure and those certified.

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