

# Analysis of Copper in Biscuits and Bread Using a Fast-Program Slurry Electrothermal Atomic Absorption Procedure

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A rapid method for the electrothermal atomic absorption determination of copper in biscuit, bread, and cereal samples is reported. Suspensions were prepared from ground samples in a 20% ethanol/water solution containing 0.5% nitric acid and directly injected into the atomizer. The furnace conditions were optimized using fast-program methodology by replacing the drying and ashing steps with a modified drying stage. Atomization was performed at 2500 °C, and no clean-out was necessary. Calibration was performed with aqueous standards. The results obtained by the slurry procedure were similar to those obtained by conventional calcination and dissolution in acids. Results for two standard reference materials are also reported.

## INTRODUCTION

Electrothermal atomization atomic absorption spectrometry (ETAAS) is currently used in many laboratories for the routine determination of small amounts of metals in foods. The conventional practice of ETAAS requires complete dissolution of solid samples to obtain a solution suitable for measurement. Since the dissolution stage is frequently tedious and prone to contamination or analyte loss, different procedures have been developed for the direct analysis of solid samples. These methods have been recently reviewed (Bendicho and de Loos-Vollebregt, 1991). One of the proposed approaches uses, instead of a solution, a suspension prepared from finely ground sample. Such suspensions or slurries, if adequately prepared, can be used in the same way as the solutions, and the determinations can be carried out using commercially available spectrometers. Since the time-consuming dissolution step is avoided, the procedure is considerably shortened and the risks of contamination or loss are minimized.

In the present study, a slurry ETAAS approach has been developed to determine the copper content of a variety of biscuit, bread, and cereal samples. To obtain the simpler, faster procedure, the so-called fast-program methodology (Bahreyni-Toosi and Dawson, 1983; Halls, 1984, 1989; Halls et al., 1987; Hinds et al., 1991) was also considered. This approach, which is based on the simplification of the furnace program by replacing the drying and ashing steps with a modified drying stage, shortens the time necessary to run the heating program.

## EXPERIMENTAL PROCEDURES

**Apparatus.** A Perkin-Elmer Model 1100B atomic absorption spectrometer, equipped with deuterium arc background correction, and an HGA-400 electrothermal atomizer were used. Measurements were performed at 324.8 nm using a hollow cathode lamp operated at 15 mA and a bandwidth of 0.7 nm. Argon was used as the inert gas at 300 mL/min except in the atomization step, where the flow was stopped. Pyrolytic graphite coated tubes were obtained from Perkin-Elmer (Part B013-5653). Wall atomization was used. The temperature changes in the drying step were studied using an iron-constantan (type J) thermocouple obtained from Herten, S.L. (Castellón, Spain). The output of the thermocouple was connected to a personal computer by means of a PCLab 818PG data acquisition card (Advantech Co.). The

Table I. Furnace Program for the Copper Determination

step	temp, °C	ramp, s	hold, s
dry	200	1	20
atomize <sup>a</sup>	2500	0	3

<sup>a</sup> The argon flow was stopped.

response time of the device to a change from 20 to 500 °C was estimated to be about 5 s. A Branson ultrasonic bath of 14-W constant power was also used. To estimate the particle size distribution, an Imco 10 image computer (Kontron) and a set of nylon sieves were used.

**Reagents.** Doubly distilled water was used throughout. A stock solution of copper (1000 µg/mL) was obtained from Panreac (Spain). High-quality ethanol (Riedel-deHaën), nitric acid (Merck), and hydrochloric acid (Fluka) were used.

**Procedures.** The samples were first dried and then ground in a domestic mill for 5 min. No sieving was carried out. Slurries were prepared by adding 25 mL of a 20% v/v ethanol/water solution containing 0.5% nitric acid to the ground sample (typical amounts ranged 50 to 200 mg). The suspensions were submitted to ultrasounds for 5 min and then magnetically stirred for another 10 min. While the suspensions were being continuously stirred, 20-µL aliquots were taken and injected into the furnace. The heating program given in Table I (where the quoted temperatures are values set on the HGA-400 power supply) was run and the background corrected peak area due to copper obtained. Calibration was performed using aqueous standards. Certified reference materials were analyzed in the same way. In this case, the samples were not submitted to drying and grinding, but a correction for the moisture content was done using separate fractions.

The samples were previously analyzed for comparison purposes. For this, fractions of 0.5 g of the dried samples were calcined at 500 °C for 8 h in porcelain crucibles. The ashes were treated with 5 mL of hydrochloric acid and 1 mL of nitric acid and heated to almost dryness; the solution was finally diluted up to 50 mL. A 10-fold dilution was carried out, and aliquots of 20 µL were injected into the furnace.

For the particle size distribution study, aliquots of 0.2% biscuit suspensions were extended over poly(L-lysine)-coated histological slides and air-dried. The particles were stained with an iodine solution for 1 h and observed through an axioskop Zeiss microscope connected to the image analyzer. Another estimation of the particle size distribution was obtained by means of wet sieving, drying, and weighing the fraction of sample collected on each sieve.

## RESULTS AND DISCUSSION

Holcombe and Majidi (1989) have discussed in depth the errors associated with the sampling of suspensions,

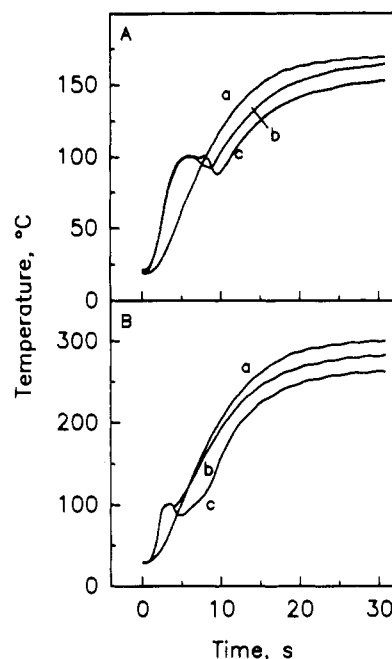
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concluding that errors are minimized when concentrated slurries are prepared from solids with a low particle size and a narrow particle size distribution. When ETAAS is used, the condition of a low particle size is much less severe than for the case of flame atomization, due to the relatively high residence time of the solid particles inside the atomizer. Thus, it has been proved (Miller-Ihli, 1988, 1993) that successful analyses can be obtained for a variety of samples even when using particles with a diameter as large as 500  $\mu\text{m}$ . The same author pointed out that the density of the solid sample must also be taken into account. In practice, suspensions must be prepared using a volume of solid to volume of suspension liquid ratio of less than 0.25 to ensure easy and reproducible pipetting. The extensive work of Miller-Ihli has also shown that the reproducibility is much better if an autosampler equipped with an ultrasonic slurry sampler is used (Miller-Ihli, 1989, 1992, 1993). The use of concentrated slurries is adequate for samples whose physical characteristics make it difficult to decrease the particle size by grinding. If the slurry is too concentrated, in addition to the need to use alternate lines to obtain measurements within the linear response range, there is a risk of unvolatilized residues accumulating inside the atomizer. Thus, although a low particle size is not an essential condition, it is recommended when one is dealing with solids whose particle size can be decreased in a simple way such as a mild grinding stage. The benefit of a reduced particle size can be especially appreciated when an autosampler is not available.

A number of previous experiments were devoted to the study of sample comminution and the methodology by which to obtain appropriate slurries. The use of a ball mill was proven to be inadequate due to the physical-chemical characteristics of biscuits; therefore, a domestic mill was used to triturate the samples. The ground samples were suspended in different media, and 20- $\mu\text{L}$  aliquots were submitted to electrothermal atomization. The relative standard deviation (RSD) of the signal obtained from copper was used to judge the quality of the approach. The direct suspension in pure water led to a poor reproducibility, as the solid particles rapidly settled out. The addition to the suspending medium of chemicals such as Triton X-100, poly(vinyl alcohol), glycerol, or detergents did not improve the slurry stability. The best results were found when a 20% v/v ethanol/water solution was used. Since only a partial stabilization was achieved, the slurries were hand-pipetted while the suspensions were being magnetically stirred. This was done in this way due to the lack in our laboratory of an autosampler equipped with ultrasonic mixing accessory that, if available, is strongly recommended.

**Optimization of the Furnace Program.** Conventional ETAAS methodology requires consecutive steps of drying, ashing, and atomization of the samples. However, Halls (1984) proved that the drying and ashing steps can be replaced by a modified drying stage, thus introducing a considerable shortening of the furnace program time. The furnace conditions were optimized using this fast-program methodology.

For the optimization of the modified drying step conditions, following Hall's suggestion, a 1-s ramp time was selected. The drying temperature ( $T_d$ ) and the drying time ( $t_h$ ) to be programmed in the HGA-400 power supply were experimentally studied by measuring the temperature of the wall of the graphite tube with a thermocouple. Figure 1 shows the results obtained when the tip of the thermocouple was placed on the wall of the pyrolytic tube through the injection hole. Curves a represent the profiles obtained when no slurry was introduced into the tube, and curves b and c correspond to 0.1 and 0.8%, respectively,

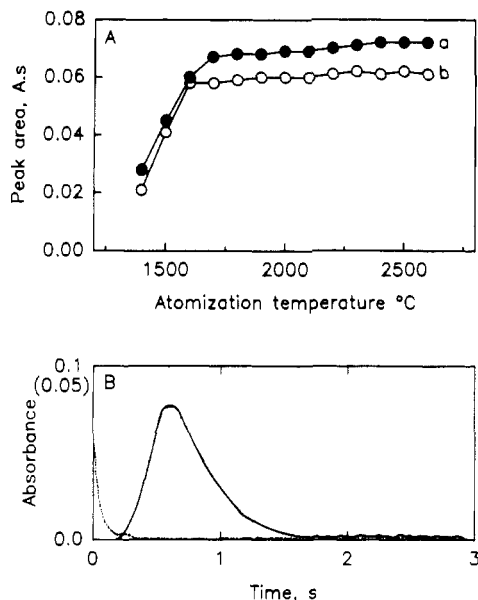


**Figure 1.** Temperature response of the thermocouple in the drying step. Drying temperature: (A) 160 °C; (B) 300 °C. Curves a correspond to no slurry injected, and curves b and c are the profiles obtained for 25- $\mu\text{L}$  injections of 0.1 and 0.8% slurries, respectively.

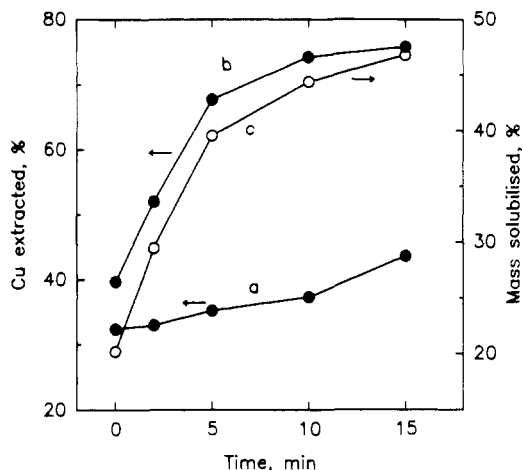
slurry concentrations. In all cases, 25  $\mu\text{L}$  of the sample was injected. Drying of the sample was completed when a plateau near 100 °C was obtained. As can be seen, drying was performed in 10 and 4 s for a 0.1% slurry when  $T_d$  temperatures of 160 and 300 °C, respectively, were used. Liquid sputtering was noted for temperatures higher than 200 °C. At this temperature, hold times ranging from 7 to 11 s approximately, depending on the slurry concentration, allowed complete sample drying. However, as the electrothermal atomizer used switches automatically to the reduced or null gas flow condition 5 s before the beginning of the atomization step, it was necessary to increase the drying time to ensure that the gas had completely removed the smoke before the atomization step. Thus, drying was achieved with good reproducibility at 200 °C in a single step of 20 s. The analysis was found to be possible without an ashing stage.

The atomization temperature was optimized for both a standard solution containing 4 ng/mL of copper and a 0.2% slurry prepared from a biscuit containing 1.8  $\mu\text{g/g}$  of the analyte. Results are summarized in Figure 2A. No significant differences were detected between the optimal atomization temperatures for liquid standards and slurries. The maximum signal was reached above 1700 °C. The atomization profiles showed a tailing effect at low atomization temperatures; therefore, a 2500 °C temperature was selected as optimal. An additional advantage of the use of a high temperature is that a clean-out step can be avoided, thus simplifying the furnace program. Figure 2B shows a typical atomization profile obtained from a suspension using the recommended conditions. It should be noted that under the conditions here studied no significant buildup of carbonaceous residues inside the tube was noted and only occasionally a mechanical cleaning of residues was necessary.

**Particle Size. Reproducibility.** To estimate the particle size distribution, an image analyzer was first used, as indicated under Experimental Procedures. The data thus obtained indicated a low particle size. However, as these data did not agree with the poor reproducibility found when using suspensions with low percentage of solid, they



**Figure 2.** (A) Effect of the atomization temperature on the peak area: (a) 4 ng/mL copper standard solution; (b) 0.2% biscuit slurry. (B) Atomization profile obtained from a 0.2% biscuit slurry.



**Figure 3.** Extraction of copper into the liquid phase: (a, c, and d) results for a sample suspended in a medium with 0, 0.2, and 0.5% nitric acid, respectively; (b) results for a suspension prepared from the 12–20- $\mu\text{m}$  fraction using no nitric acid in the suspension medium.

were considered to be unreliable. A more reliable estimation was made by using wet-sieving, which showed that the bulk of the particles was below 150  $\mu\text{m}$ .

It has been demonstrated (Miller-Ihli, 1988; Bradshaw and Slavin, 1989; Epstein et al., 1989) that the ultrasonic treatment and the solubilizing action of the suspending medium result in a fraction of the analyte being extracted into the supernatant. An increase of the extraction degree causes an increase in reproducibility, because sampling problems are minimized. The percentage of copper extracted into the liquid phase was studied using a biscuit suspension and a suspension prepared from a fine fraction (12–20  $\mu\text{m}$ ) obtained by sieving the same sample. After ultrasonic treatment, the suspensions were magnetically stirred, and 1-mL aliquots were taken at different times and filtered through membrane chromatographic filters. The determination of copper in the filtrates permitted the estimation of the analyte fraction extracted into the liquid phase. The results, which are summarized in Figure 3, demonstrate that the extraction efficiency increased with stirring time and was more effective for the fraction containing the smallest particles. The presence of nitric

**Table II.** Effect of the Suspension Concentration on the Reproducibility ( $n = 10$ , 20  $\mu\text{L}$  Injected)

slurry, % (m/v)	Cu taken, ng	RSD, %		
		total	instrum	slurry samp
Direct Suspension in a 20% (v/v) Ethanol Medium				
0.04	0.014	23.9	12.7	20.2
0.32	0.115	8.1	3.0	7.5
0.48	0.173	4.4	1.3	4.2
0.75	0.270	4.4	1.2	4.2
1.01	0.363	2.4	1.2	2.1
1.44	0.518	2.1	1.0	1.8
Direct Suspension in a 20% (v/v) Ethanol + 0.5% HNO <sub>3</sub> Medium				
0.04	0.014	14.5	12.7	7.0
0.32	0.115	4.6	3.0	3.5
0.48	0.173	1.9	1.3	1.4
0.75	0.270	1.8	1.2	1.3
1.01	0.363	1.7	1.2	1.2
1.44	0.518	1.6	1.0	1.2
Suspension of Ground Carbonaceous Residues in a 20% (v/v) Ethanol Medium				
0.01	0.023 <sup>a</sup>	10.0	8.3	5.6
0.035	0.080 <sup>a</sup>	3.9	3.5	1.7
0.075	0.170 <sup>a</sup>	1.8	1.3	1.2
0.108	0.245 <sup>a</sup>	1.6	1.3	0.9
0.142	0.322 <sup>a</sup>	1.4	1.2	0.7
0.202	0.458 <sup>a</sup>	1.1	1.0	0.5

<sup>a</sup> Mass of sample/mass of dry ashed sample = 6.3.

acid in the suspension media considerably increased the percentage of copper extracted into the liquid phase (curves c and d). Concentrations of nitric acid higher than 2% led to a copper recovery of close to 100%. However, to increase the useful lifetime of the pyrolytic material, a 0.5% nitric acid concentration is recommended. Under this condition about 35% of the solid mass was dissolved by the action of the suspension media.

The reproducibility was studied using suspensions prepared with different percentages of solid, and the results were compared with those obtained for replicate measurements of aqueous standards containing the same concentrations of analyte as has been recommended (Miller-Ihli, 1993). In this way, the precision due to the slurry sampling alone was obtained by subtracting the instrument precision ( $\sigma_{\text{instrum}}^2$ ) from the total slurry measurement precision ( $\sigma_{\text{total}}^2$ ). Table II summarizes the results obtained for 10 injections of slurries prepared using different experimental conditions. As could be expected, the precision notably increased when nitric acid was added to the suspension media. For suspensions prepared with low percentages of solid the presence of large particles produced poor results, though the RSD values again decreased when nitric acid was present due to the extraction effect.

Table II includes, for comparison purposes, data obtained using a different approach. A number of samples were submitted to a mild calcination stage (500 °C for 1.5 h) in an oven to achieve a more effective sample comminution. The carbonaceous residues thus obtained were ground in a ball mill, and suspensions were prepared from these powders. A similar approach has already been reported (Ebdon et al., 1990; López García et al., 1992) to overcome matrix effects and facilitate sample comminution. Using these experimental conditions, the grinding of the carbonaceous residues was highly efficient and 100% of copper was extracted into the supernatant, even with no nitric acid in the suspension media. For these reasons the precision due to the slurry sampling was very high (Table II). However, the improvement in reproducibility does not justify the time spent in the precalcination stage. If an autosampler with slurry sampling accessory is not

**Table III. Slopes of the Standard Addition Calibration Graphs<sup>a</sup>**

sample	slope (mean $\pm$ SD)
aqueous standards	0.7028 $\pm$ 0.0692
biscuit suspension	0.7243 $\pm$ 0.0512
bread suspension	0.7186 $\pm$ 0.0583

<sup>a</sup> Each graph was constructed from five points. Each point was measured four times.

**Table IV. Copper Content in Different Samples**

sample	Cu, $\mu\text{g/g}$ (mean $\pm$ SD)	
	slurry procedure	reference procedure
biscuit 1	1.22 $\pm$ 0.049	1.27 $\pm$ 0.050
biscuit 2	1.05 $\pm$ 0.044	1.10 $\pm$ 0.003
biscuit 3	1.06 $\pm$ 0.034	1.08 $\pm$ 0.046
biscuit 4	1.45 $\pm$ 0.057	1.37 $\pm$ 0.055
biscuit 5	1.51 $\pm$ 0.099	1.44 $\pm$ 0.045
biscuit 6	1.36 $\pm$ 0.032	1.32 $\pm$ 0.096
biscuit 7	1.25 $\pm$ 0.053	1.22 $\pm$ 0.045
biscuit 8	1.39 $\pm$ 0.093	1.48 $\pm$ 0.053
biscuit 9	1.29 $\pm$ 0.034	1.25 $\pm$ 0.096
whole meal bread	1.95 $\pm$ 0.120	2.13 $\pm$ 0.033
white bread 1	1.94 $\pm$ 0.108	2.09 $\pm$ 0.130
white bread 2	2.00 $\pm$ 0.069	1.90 $\pm$ 0.050
white bread 3	1.94 $\pm$ 0.123	1.91 $\pm$ 0.143
soft bread	2.04 $\pm$ 0.068	2.08 $\pm$ 0.083
breakfast cereals	2.96 $\pm$ 0.148	2.94 $\pm$ 0.158
rice flour (SRM 1568a)	2.33 $\pm$ 0.140	2.40 $\pm$ 0.3 <sup>a</sup>
wheat flour (SRM 1567a)	2.16 $\pm$ 0.030	2.10 $\pm$ 0.2 <sup>a</sup>

<sup>a</sup> Certified values.

available, this approach could be considered as an alternative to direct suspension for samples which can only be suspended and sampled with difficulty using hand-pipetting. No practical problems were detected with the samples here studied, and the fast direct suspension method is recommended. Taking into account the levels of copper (1–3  $\mu\text{g/g}$ ) in the samples, suspensions must be prepared with percentages of solid above 0.3%. Under this condition, even when nitric acid is absent from the suspension media, the precision due to the slurry sampling can be considered to be adequate for routine purposes.

**Calibration and Comparison of Results.** Standard addition calibration graphs were obtained for both a slurry prepared from a biscuit and a suspension prepared from a bread sample. The slopes of these graphs (Table III) were very close to that of an ordinary calibration graph obtained from aqueous standards under identical experimental conditions. They indicate the absence of matrix effect and suggest that the simplest calibration with aqueous standards must be valid. The reliability of the procedure was assessed by the determination of copper in samples of biscuit and bread using the recommended methodology. Table IV compares the results obtained with the proposed method and those obtained with a conventional procedure based on dry-ashing followed by dissolution in acids. No statistically significant differences were observed between these results using a least-squares fitting. The reliability of the procedure was further confirmed by the analysis of two standard reference materials. Again, no significant differences were observed between the certified copper contents and those experimentally found.

**Conclusion.** In the present study, a method for the determination of copper in biscuit and bread samples avoiding the sample dissolution step is reported. The direct suspension of the powdered samples in an ethanol/water mixture allows copper determination by ETAAS. This procedure represents a considerable saving of time over the conventional dry-ashing followed by acid dissolution procedure. The reproducibility and accuracy of

the results for 15 samples suggests that the procedure could be useful for routine purposes even when using hand-pipetting of the slurries, although the use of an autosampler with a slurry sampling accessory is recommended.

#### ACKNOWLEDGMENT

We are grateful to the Spanish DGICYT (Project 90-0302) for financial support and to Dr. M. T. Castells (Study and Image Analyzer Unit, University of Murcia) for the analysis of particle size distribution. N.C. acknowledges a fellowship from Consejería de Cultura, Comunidad Autónoma de la Región de Murcia (Spain).

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Received for review December 21, 1992. Revised manuscript received July 16, 1993. Accepted July 26, 1993.\*

\* Abstract published in *Advance ACS Abstracts*, September 15, 1993.