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DETERMINATION OF TRACE METALS IN FISH TISSUE BY FLAME ATOMIC ABSORPTION SPECTROMETRY USING A DISCREET NEBULIZATION TECHNIQUE

KEY WORDS: trace metals, fish tissue, atomic absorption, discreet nebulization

Gerald J. Ramelow and Turgut İ. Balkaş

Department of Marine Science
Middle East Technical University
Ankara, Turkey

ABSTRACT

Flame atomic absorption spectrometry is applied to the analysis of trace metals in fish tissue after digestion of the sample with nitric acid in a decomposition vessel. The digested sample is analyzed directly using a discreet nebulization technique. Enhancement effects were observed for all elements studied. The method was applied to the analysis of copper, iron and zinc in several species of fish.

INTRODUCTION

The analysis of trace metals in fish is of importance in pollution monitoring[1]. Relatively large concentrations can conveniently be determined by conventional flame atomic absorption while extremely low concentrations are commonly determined

by flameless furnace methods. However, the natural levels of some elements are often such that conventional flame atomization, without some type of pretreatment such as chelation and extraction into an organic solvent[2-4], will not provide adequate sensitivity.

Flameless methods, although without doubt very sensitive, may not always be available or may not be preferred due to their higher cost per analysis and other well-known disadvantages such as greater time per analysis and the need for considerable operator skill.

Recently a new type of sampling for flame atomic absorption spectrometry has been described[5-12] in which discreet amounts of sample are nebulized into the flame. It is especially advantageous when analyzing solutions with a high dissolved solids content as such solutions can rapidly clog the nebulizer-burner when using continuous nebulization. This technique is also useful when the sample volume is limited.

A promising application of this technique is in the analysis of trace metals in fish tissue and other marine samples after acid digestion. The high solids matrix and the strong acidity of the digested sample do not allow the use of the standard nebulizer-burner with continuous nebulization. With the discreet nebulization technique it should be possible to determine several elements in a single digested sample.

In this paper we describe the application of the injection technique to the analysis of copper, iron and zinc in fish tissue

after acid digestion. The use of a glass nebulizer with the acidic digests is also discussed.

EXPERIMENTAL

Instrumentation

The basic instrument used was a Varian-Techtron Model AA-6 atomic absorption spectrophotometer equipped with automatic gas control and a Model A-25 recorder. The standard single-slot air-acetylene burner head was used. Varian Cu-Fe-Mn-Cr-Ni-Co multi-element and Zn single-element hollow cathode lamps were operated at or below the manufacturer's recommended operating currents.

Optimum gas flow rates and burner positions were determined while aspirating continuously standard solutions of the elements.

To analyze the strongly acidic digested sample solutions, a nebulizer was constructed entirely of glass which could be fitted into the standard nebulizer holder and had approximately the same flow rate characteristics as the standard metal nebulizer. A plastic micropipet tip which acts like a funnel was attached to the nebulizer as shown in figure 1.

The sample aliquots were injected into the funnel with an adjustable micropipet (Finnpipette).

Experimental data were evaluated with a Wang Model 600 minicomputer-calculator.

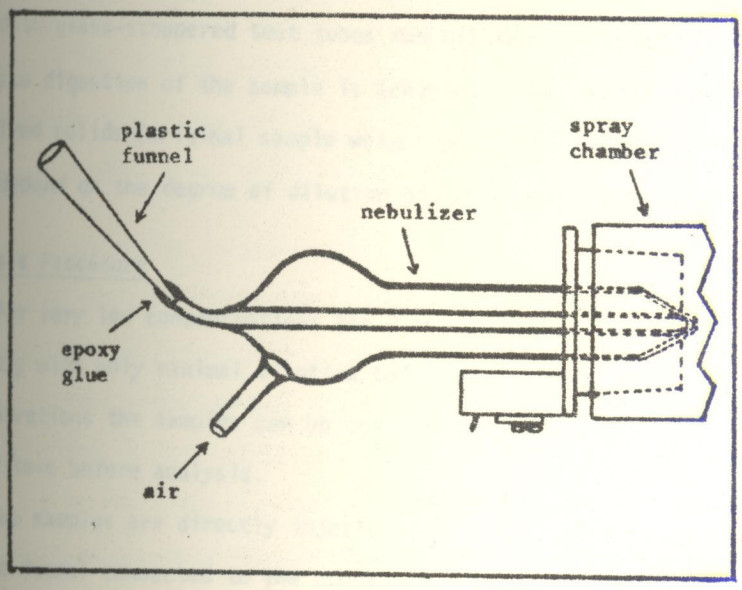

FIG. 1

Diagram of glass nebulizer shown mounted in place of standard nebulizer (not drawn to scale).

Reagents

Stock solutions of the elements (1 gram/liter) were prepared from pure metals or reagent-grade chemicals.

The nitric acid used in the digestions was obtained from Merck and showed a detectable blank only for iron.

Distilled, deionized water was used throughout.

Digestion procedure

The usual procedure is as follows: 1 or 2-gram samples (fresh weight) are digested with 2 to 4 ml concentrated nitric acid in digestion bombs (Uniseal Decomposition Vessels, Ltd.)

at 150°C for 1.5 hours. After digestion the samples are transferred to glass-stoppered test tubes and diluted to minimal volume. Complete digestion of the sample is achieved. The percentage of dissolved solids (original sample weight per final diluted volume) will depend on the degree of dilution of the sample after digestion.

Analysis Procedure

For very low concentrations the digested sample can be analyzed directly with only minimal dilution to a known volume. For higher concentrations the samples can be conveniently diluted to 10 or 20 milliliters before analysis.

The samples are directly injected with a micropipet into the plastic funnel connected to the nebulizer. It is important to reproducibly inject the sample just as with furnace atomizers. The absorption is seen as a sharp peak which can conveniently be measured using the peak read facility of the instrument.

RESULTS AND DISCUSSION

Effect of injection volume

It has been well documented that the absorption signal increases with injected volume until a value equivalent to the continuous signal is obtained. In the case of the standard Varian nebulizer, this volume was found to be about 150 µl with small variations depending on the element being studied.

The glass nebulizer constructed in our laboratory required, on the other hand, a volume of 400-500 µl due to its high consump-

tion rate (about 40 ml/min). This relatively large volume cancels some of the advantages to be gained from the injection technique. However, the problem will be solved with the construction of a glass nebulizer whose uptake rate more closely resembles that of the standard metal nebulizer.

Performance of the glass nebulizer

The glass nebulizer described in this work, although it did not achieve the sensitivity of the standard metal nebulizer, has nevertheless proved proved valuable for the direct determination of trace metals in highly acidic solutions resulting from the digestion of fish tissue. Such a nebulizer can be purchased commercially or can be constructed by any competent glassblower. However, as discussed in the previous section, the nebulizer should be compatible with the flow-rate characteristics of the instrument, not only for sensitivity considerations, but also so that rapid interchange of nebulizers can be made without major modification of the instrument.

Enhancement effect of fish digests

The accuracy of the direct injection technique was studied by spiking swordfish samples (dissolved solids content - 20%) with copper, iron and zinc to give final concentrations ranging from 1 to 5 µg/ml. The average recovery, calculated from calibration curves prepared with aqueous standards, was 116%. This enhancement effect was seen to be independent of the species as shown in Table 1.

TABLE 1

Enhancement Effect of Fish Digests*

Species	% Enhancement	
	Cu	Fe
Swordfish	103	111
Bonito	104	112
Shrimp	107	109

*Samples (10% dissolved solids) spiked to give added Cu and Fe concentrations of 5 µg/ml, 40 % acidity.

This effect was shown not to be due to non-atomic absorption in the flame by checking it with a hydrogen lamp background corrector. The effect of acidity was also studied using aqueous standards and a strong depressive effect was observed for each of the elements.

To obtain accurate results, therefore, it is necessary to eliminate this matrix effect, either by using a type of microstandard additions technique[5, 13] or to prepare the calibration standards in the same matrix as the samples.

Analysis of fish and other marine organisms

Several different species of fish and other marine organisms were analyzed for copper, iron and zinc using the techniques described. A composite fish digest was used to prepare the calibration standards after subtracting the blank value of each element from all readings.

An acid blank digested in the same manner as the samples gave only negligible readings. The results given in Table 2 illustrate the wide concentration ranges which can be analyzed by this technique.

CONCLUSION

The direct injection of acidic fish digests into the flame of an atomic absorption spectrophotometer using a glass nebulizer

TABLE 2

Analysis of Marine Organisms[§]

Species	µg/g (fresh wt.) Cu	Fe	Zn
Swordfish	1.2	8.1	9.7
Striped mullet	0.2	0.7	7.4
Bonito	1.3	6.8	7.9
Mackerel	1.0	5.9	9.6
Crab	7.0	6.4	24.9
Shrimp	2.9	1.6	12.5
Mussel (small)[†]	1.7	151.0	23.7
Mussel (medium)[†]	1.2	21.3	10.8
Mussel (large)[†]	1.8	24.0	9.2

[§]20 % dissolved solids, 40 % acidity.

[†]Size arbitrarily determined on basis of total fresh weight and shell length.

is a useful method for the determination of trace levels of several elements in fish and other marine organisms, especially at levels which would not normally be detectable with conventional nebulization after dilution of the sample. The method should also be useful for the analysis of other types of foodstuffs.

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