Spectrochimica Acta, Vol. 33B, pp. 489 to 494

0031-6987/78/0801-0489$02.00/0

Non-flame atomic absorption analysis of trace metal elements fixation by seric proteins of crustacean decapods*†

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(*Received* 27 *February* 1978)

Abstract—Metals fixed by seric proteins of crustacean decapods were determined by non-flame (Cu, Pb, Fe) or flame (Zn) atomic absorption spectrometry after separation of the proteins by electrophoresis. The electrophoregrams were cut into 5 mm fractions and each fraction was dissolved in nitric acid. The solutions thus obtained were analyzed. Metal concentrations of the order of a few ppm in protein fractions were covered.

1. INTRODUCTION

AT PRESENT, one is more and more concerned with marine environment problems and particularly with sea water pollution. The concentrations of the main trace elements in sea water are according to VALKOVIC [1]: Cr—0.2, Mn—2, Fe—3.4, Co—0.3, Ni—5.5, Cu—3, Zn—10, As—3, and Pb—0.03 (μg/l).

Along the coasts, in the vicinity of industrial sewage areas and estuaries of rivers carrying industrial effluents, the trace element concentrations may increase up to several tens μg/l. This tremendous increase has generally important effects on the metabolism of plants and animals living in these waters. The present paper describes an experimental procedure allowing the quantification of heavy metals fixation by seric proteins of some crabs.

2. EXPERIMENTAL PROCEDURES

2.1. *Samples*

Experiments have been performed with three species of marine crab: chiefly *Macropipus puber* and, to a lesser extent, *Carcinus maenas* and *Cancer pagurus*. Specimens were collected near Roscoff (Brittany). Blood was collected from the walking legs sinus by means of a syringe and kept at room temperature for 24 h. With two of these species, *M. puber* and *C. maenas*, blood clotting may occur in the meantime. In these cases, the clots were thoroughly broken with a glass rod. Hemolymphs were centrifuged at 6000 rpm for 10 min. The more or less deep blue coloured supernatant liquid corresponds to serum.

2.2. *Addition solutions*

Addition solutions were prepared to determine metals fixation. The addition solutions contained 35 g/l NaCl and 0.25 g/l of one of the analytes, viz. iron (II) as $FeSO_4$, iron (III) as $Fe_2(SO_4)_3$, lead as $Pb(NO_3)_2$, zinc as $ZnSO_4$ or copper as $CuSO_4$.

For each metal, 100 μl of the addition solution was added to 400 μl serum. The concentration of the added metal then is higher than the average concentration in the seric proteins. The electrophoretic separation was started 3 h after the addition (20°C).

2.3. *Electrophoretic separation*

In practice, the volume of the electrophorezed serum sample is 6 μl. The total protein concentration in crustacea serum ranges from 20 to 70 g/l. Starting from a 6 μl sample we thus get an amount of protein varying from 240 to 420 μg. Copper is naturally present in the crustacea serum, for it is a constituent element of their respiratory pigment, hemocyanin, which represents during an important part of the life about 95% of their total seric protein content.

* Dedicated to the memory of Professor HEINRICH KAISER.

† Paper presented at the 33th GAMS Congress, Paris, November 1977.

[1] V. VALKOVIC, *Trace Element Analysis*. Taylor, London (1975).

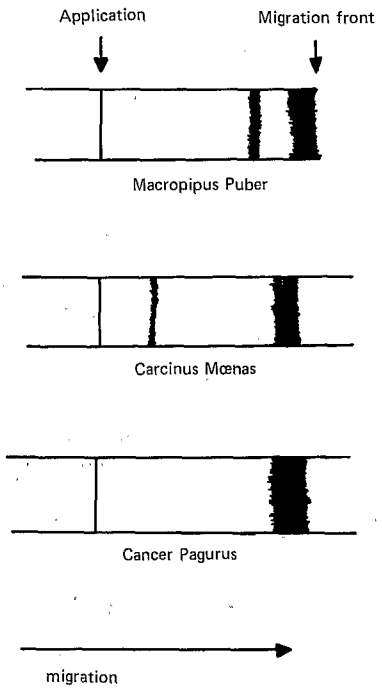

Fig. 1. Electrophoretic separation of crustacea serum proteins.

The copper concentration in hemocyanin is about 0.18%. Thus the total amount of copper corresponding to electrophoretic fractionation is approximately 0.2 to 0.5 μg. If we now try to look for other metals that may be associated with copper, their concentration will be much lower. Finally we have to determine some tens of ng of metal in a sample solution of 0.5 ml with concentrations of 0.01 to 0.1 μg/ml, i.e. we have a microdetermination of trace elements.

Each set of experiments covered:

(i) a blank test;
(ii) a sample fractionation with amide black to locate protein bands (see Fig. 1);
(iii) a sample fractionation for metal analysis.

Seric proteins were fractionated on cellulose acetate strips (17 × 2.5 cm cellogel SEBIA) in pH 6.4 potassium phosphate buffer. Under these conditions there is no dissociation of the respiratory pigment.

The experimental conditions were as follows:

—phosphate buffer: 1 M NaOH solution is added to 500 ml 0.1 M KH_2PO_4 to adjust pH to 6.4; then the solution is made up to 1000 ml;
—sample solution: 6 μl;
—migration time: 2 h;
—voltage: 200 V;
—current: 20–30 mA.

2.4. *Preparation of sample solutions*

After electrophoresis, strips (i) and (iii) were cut perpendicularly to their axis in 0.5 cm wide narrow bands. Each band was dissolved in a Teflon bomb with 0.5 ml of 10% suprapur nitric acid. The bomb was shaken in an ultrasonic agitator. The metal recovery at the end of these treatments is more than 85%.

2.5. *Analysis*

Copper, lead and iron were determined by non-flame atomic absorption spectrometry (AAS) using a sample size of 10 μl. Zinc was determined by flame-AAS using a sample size of 200–500 μl.

2.5.1. *Zinc determination.* The zinc concentration in the electrophoretic fractions varies between 50 and 100 μg/l. This is above the range of non-flame AAS (0.1–1 μg/l). We have studied a microanalytical method using the flame atomizer. Exactly measured sample solutions of 200 or 500 μl are nebulized with a device as shown in Fig. 2. The atomic absorption is recorded as a peak because the atomization lasts less than one second. The detection limit in complex media is 0.02 μg/ml Zn. The analytical range is 0.05–0.5 μg/ml and the precision is about 5%.

There are no interferences because mineral constituents of the serums are eliminated in the electrophoretic treatment, while seric proteins themselves do not give interference.

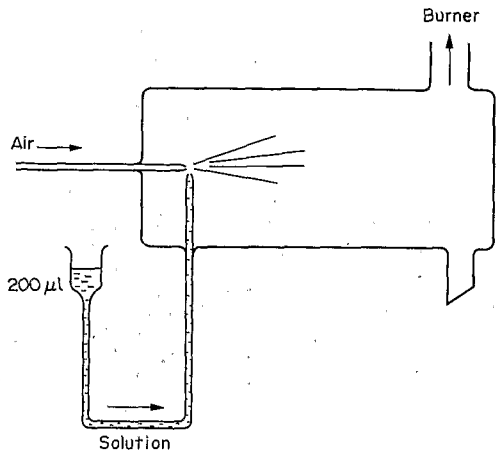

Fig. 2. Device for microanalysis by flame AAS.

2.5.2. *Lead determination.* Sample solutions are atomized by electrothermal atomization in a graphite furnace. The thermal program depends on the sample matrix and is studied for each metal separately. The temperatures and times of decomposition and atomization are determined according to the "double curves method" [2–6]: two curves of the peak height absorbance signal versus temperature are plotted (Fig. 3). The first curve is a plot of the signal at the optimum atomization temperature vs the decomposition temperature as independent variable; the second curve is a plot of the signal vs the atomization temperature as independent variable. The first curve gives the temperature at which the sample can be thermally decomposed without loss of analyte. For this study the sample is a protein solution to which lead nitrate in nitric acid has been added so that the lead concentration is 0.01 µg/ml. The volume of the solution to be atomized is 10 µl (0.1 ng Pb).

In practice, the lead determination in nitric acid solution is difficult: lead nitrate is unstable and this may cause loss of analyte. As the melting point of lead is low (327°C), it is not possible to thermally eliminate the matrix completely. A non-specific signal can be recorded and part of the analyte may be lost. A possible remedy is the addition of phosphoric acid to the sample in the furnace (10 µl 0.1% H_3PO_4). Lead phosphate is less volatile than lead (melting point: 1014°C) and more stable than lead nitrate.

The sensitivity is increased threefold with gas stop during the atomization stage. The eventual thermal program for the lead determination in electrophoretic fractions is shown in Table 1.

2.5.3. *Copper and iron determinations.* The analytical conditions are similar to those described for lead and the experimental conditions are listed in Table 1.

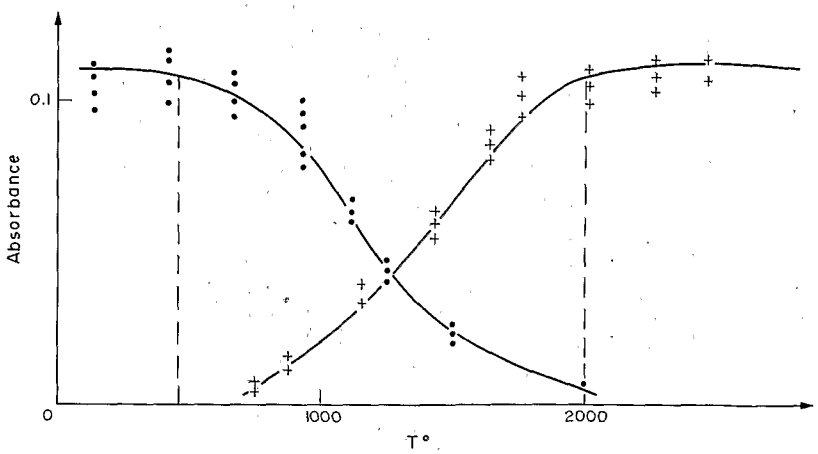

Fig. 3. Decomposition and atomization curves of lead in proteins.

[2] C. Caillot, thèse 3e Cycle, Université de Paris VI (1974).
[3] M. Pinta and C. Riandey, *Analusis* **3**, 86 (1975).
[4] C. Riandey, P. Linhares and M. Pinta, *Analusis* **3**, 303 (1975).
[5] M. Pinta, *Modern Method for Trace Element Analysis.* Ann Arbor Science Publishers, Ann Arbor, MI (1977).
[6] D. Baron, thèse 3e Cycle, Université de Paris VI (1977).

Table 1. Experimental conditions for the non-flame AAS determination of Pb, Cu and Fe

	Pb	Cu	Fe
Sample size (μl)	10	10	10
Additive	10 μl $0.1\%H_3PO_4$	—	—
Drying,			
temperature (°C)	100	100	100
time (s)	35	20	20
Decomposition,			
temperature (°C)	450	900	1200
time (s)	45	45	45
Atomization,			
temperature (°C)	1950	2500	2600
time (s)	5	5	5
Detection limit (μg/l)	1	2	2
Analytical range (μg/l)	5–50	10–100	50–500

2.5.4. *General characteristics of the method.* The precision is 5% for lead (Fig. 4) and 10% for copper and iron. The principal cause of error is contamination during all analytical operations. It is necessary to work in a "clean room" without dust (elimination of 99.9% particulates > 0.4 μm), i.e. class No. 100 in the U.S.A. norm.

The sensitivity and detection limits are adequate for the present problem. Accuracy is difficult to assess because reference samples are not available. Therefore the addition method must be used as a check on the analyses of electrophoretic fractions: deviations between results according to the classical standardization method and the addition method did not exceed 6% for Pb, Cu, Fe and Zn.

3. RESULTS AND CONCLUSION

Results for each analyte are reported by curves of the element concentration in the protein fractions as a function of time or migration distance. Figure 5 shows two curves for copper in protein fractions of *M. puber*. Curve 1 is for the natural concentration of copper in hemocyanin; curve 2 results after addition of 0.0625 g/l Cu and shows two maxima corresponding to two protein bands.

Figures 6 and 7 show the relevant curves for the zinc and lead fixation on proteins. Figure 8 illustrates the comparative fixation of increasing amounts of copper. Figure 9 finally demonstrates the simultaneous action of Cu, Zn, Fe and Pb on the serum of *M.*

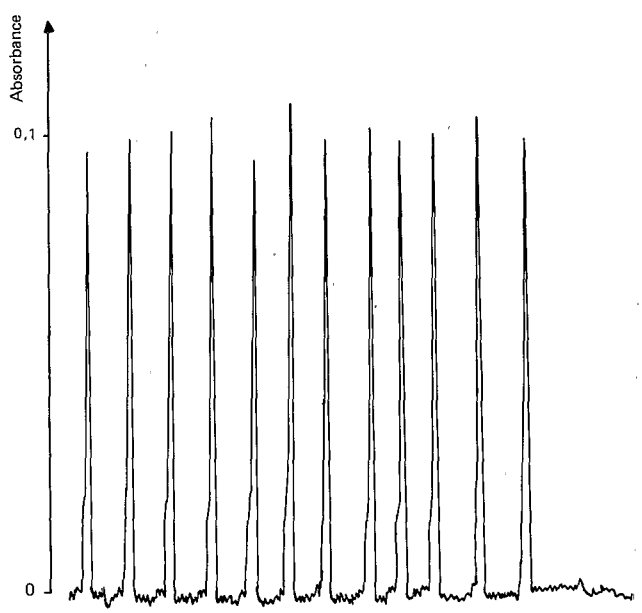

Fig. 4. Repeatability test for the lead determination.

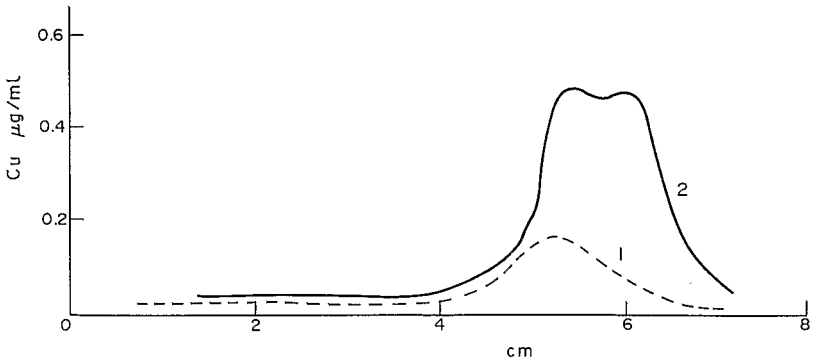

Fig. 5. Copper migration. 1—Test serum. 2—Serum after addition of 0.0625 g/l Cu.

puber: zinc is virtually not fixed, iron and lead are very weakly fixed and copper is strongly fixed on the first band in the migration front.

To conclude, non-flame AAS applied to Cu, Pb and Fe, and flame AAS applied to Zn allow the microanalytical determination of metals fixed on seric proteins of crustacea separated by electrophoresis. Metals such as Cu, Pb, Fe and Zn can be determined at concentrations as low as a few ppm in protein fractions.

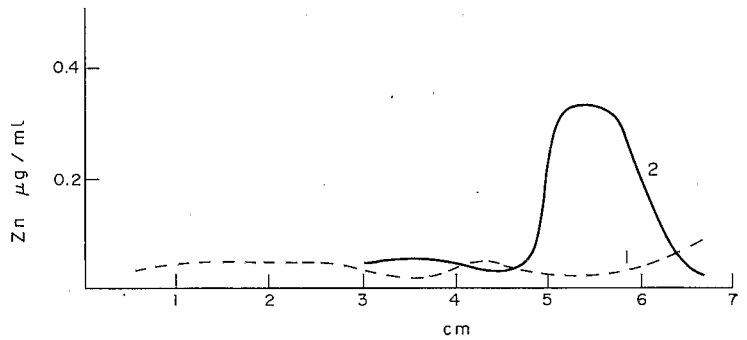

Fig. 6. Zinc migration. 1—Test serum. 2—Serum after addition of 0.0625 g/l Zn.

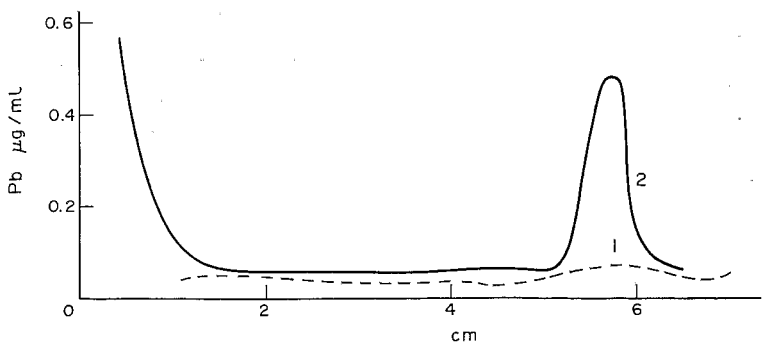

Fig. 7. Lead migration. 1—Test serum. 2—Serum after addition of 0.0625 g/l Pb.

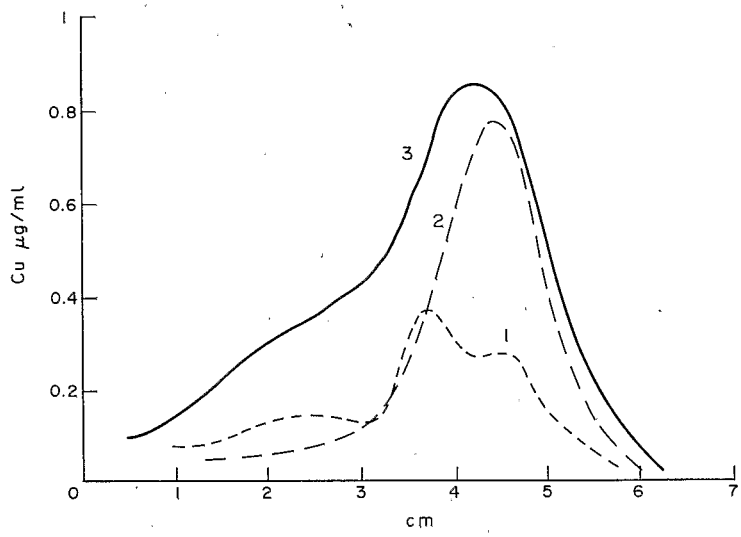

Fig. 8. Copper migration. Serum after addition of increasing amounts of copper. 1—Test serum. 2—Serum after addition of 0.0625 g/l Cu. 3—Serum after addition of 0.125 g/l Cu.

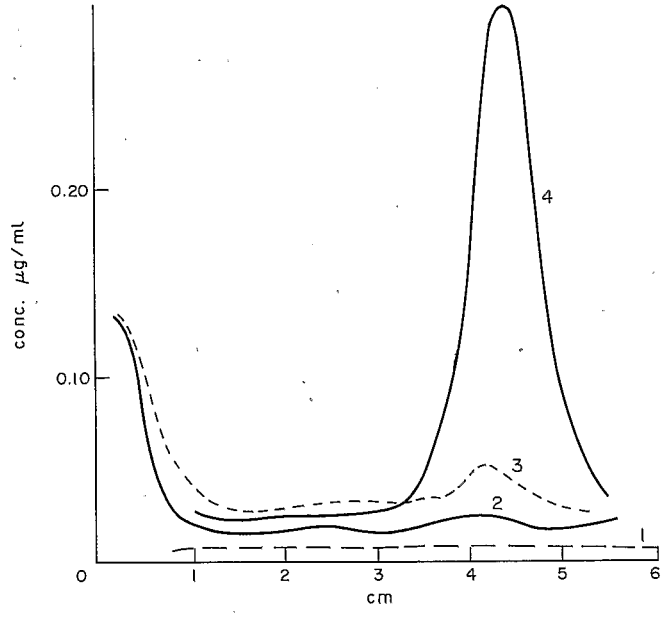

Fig. 9. Migration of various metals. Serum after simultaneous addition of Zn, Pb, Fe and Cu (0.021 g/l of each metal). Curve 1—Zn. Curve 2—Pb. Curve 3—Fe(II). Curve 4—Cu.