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Determination of Cadmium in Individual Organs and Divided Shells of Sea Water Clam by Atomic Absorption Spectrometry with a Carbon Tube Atomizer

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Cadmium contents in individual organs and divided shells of sea water clam were estimated by atomic absorption with a carbon tube atomizer. After samples were digested with nitric acid, cadmium was separated from the interfering sample matrices by extracting it into diisobutyl ketone as diethyldithiocarbamate. By this method, 0.06～0.75 ppb of cadmium was found in the divided shells. In the shellfish, cadmium was enriched to a very high extent in the kidney, to some degree in the organ of Keber.

INTRODUCTION

It has been known for many years that heavy metal contents are much higher in the marine biosphere than in the hydrosphere. Some works have indicated an increasing interest in the biosphere, particularly in relation to trace-element uptake by marine organisms.[1,2] However, the information on the trace element distribution in such an organism as marine bivalves is very few, though this may elicit considerable interest as to the impact of the marine environment on the heavy metal contents in the bivalve and as to the distribution behavior of the metal between shellfish and shell.

The atomic absorption spectrometry with a carbon tube atomizer must be very useful for the investigation of the distribution of trace elements in biological materials owing to its high sensitivity and easy procedure because some trace elements in the individual organs and especially divided shells of small bivalve are too slight to be detected by the conventional flame atomic absorption method.

Cadmium is one of the most insidiously toxic of the heavy metals to which man is exposed, but its distribution in the shellfish is not yet evident in detail.[3] In earlier papers, the estimation of cadmium in steel[4] and in waste water[5] by carbon rod atomization has been described, and previously, we also made the basic investigation for the determination of cadmium by carbon tube atomization.[6] This paper deals with the application of this technique to the estimation of cadmium in shellfish samples.

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EXPERIMENTAL

Apparatus

A Nippon Jarrel Ash flameless atomizer model FLA-10 was employed in conjunction with a Jarrel Ash model AA-1 spectrometer. The design and operation of this atomizer have been reported previously.[6] A Yanagimoto YR-101 recorder was connected to the spectrometer, and all signals were measured by the peak-height technique. The carbon tube having an inside diameter of 4.0 mm was made by boring the carbon rod (National Spectroscopic Electrode) with a length of 70 mm and with a diameter of 6.35 mm. One hole of 3.0 mm in diameter was made at the center of the carbon tube for injecting the sample solution.

Reagents

An aqueous 1% solution of sodium diethyldithiocrbamate (DDTC) was prepared daily and was shaked to remove trace metals with diisobutyl ketone (DIBK), which had been purified by shaking with 0.1 M hydrochloric acid. Standard cadmium solution was prepared by dissolving cadmium metal (99.99%) with nitric acid by evaporating to dryness, the residue being diluted with 0.1 M hydrochloric acid. Twice-distilled water was used in the preparation of all solutions.

Other chemicals were reagent-grade materials.

Procedure

The clams (metretrix lusoria) were collected at Shirahama seaside and Fukuura seaside, Hyogo Prefecture and maintained alive for a few days in tanks of sea water until dissection.

Analyses were carried out on ten individual organs for each shellfish and on several parts of each shell. The divided organs were washed in sea water, quickly rinsed with redistilled water, dried at 110°C, for 24 hrs, and weighed. Each dry sample was contained in a 30 ml conical beaker and ashed with concentrated nitric and perchloric acids by heating the beaker repeatedly on an electric hot plate. When the ashing reaction was completed, the heating at 180°C was continued to remove most of the excess inorganic acids. After allowing the beaker to cool, 0.1 M hydrochloric acid was added to dissolve any precipitate.

The procedure of the determination of cadmium by atomic absorption with a heated carbon tube atomizer was essentially the same as that described in a previous paper.[6] To the aqueous sample solution, 0.5 ml of 30% diammonium citrate was added and pH of the solution was adjusted to be in the range 8.0~8.5 with aqueous ammonia. After adding 1 ml of 0.1% DDTC, the sample solution was shaken with 1.0 or 2.0 ml DIBK for 30 min in a 30 ml separating funnel, and centrifugally separated. Ten microliters of the DIBK phase were injected into the carbon tube atomizer for measurement at 228.8 nm, and cadmium content in divided shells and shellfish was mesaured by both the calibration curve and the standard addition methods.

Shells were also divided into several pieces along the lines of growth, after the shell epidermis was removed from the shells. The divided shells were boiled with 0.01 M EDTA for an hour to remove the possibly contaminated elements, washed with redis-

tilled water, dissolved with nitric acid and treated in a similar manner as the shellfish samples except using 0.2 ml of DIBK as the organic phase. Two blanks were carried through the procedure.

RESULTS AND DISCUSSION

Preliminary Experiments

The direct determination of cadmium in shells and shellfish was difficult because of the high interference of salts and the low cadmium contents. Therefore, the extraction method of cadmium chelate with DDTC into DIBK was adopted.

The pH effect on efficiency of extraction was evaluated by extracting cadmium with 1 ml of DIBK with 10 ml of a sample solution, which had a variable pH value and which contained 1.0 ml of 1% DDTC and 0.5 ml of 30% diammonium citrate. More than 97% extraction of cadmium proceeded in the pH range 4.0~9.0. However, the effect of peak absorption of cadmium chelate in DIBK phase on the standing time was affected by the pH when the extraction was made. The stability of the chelate increased as the pH value increased and the peak absorbance of cadmium was almost invariable until 20 hrs in the pH range 8.0~8.5, though it was 20% lower at pH 6.0 and 35% at pH 4.0 after 20 hrs.

The recovery of cadmium from the sample solution into the DDTC-DIBK extraction was determined by using a radioactive tracer, cadmium–115 m. Extraction time of 30 min was used. In the extraction from about 20 ml of the sample solution of shellfish (0.3 g) into 2 ml of DIBK, the recovery of cadmium was about 99%, and in that from 20 ml and 50 ml of the sample solution of shell (3 g), the recovery was 98.2 and 96%, respectively. The effect of some foreign substances had been checked in the previous paper in detail.[6]

Cadmium contents in shell and shellfish were measured by both the calibration curve and the standard addition methods. The results (Table I) showed that both methods gave essentially the same results.

Table I. Determination of Cadmium in Shell and Shellfish by the Calibration Curve and Standard Addition Methods

	Dry Sample (g)	Cd Added (ppb)	Cd Found (ppb)	Cd in Shell (ppb)
Shell	2.131	0	2.0	2.0
	2.131	0	2.1	2.1
	2.131	2.5	4.5	2.0
	2.131	5	7.2	2.2
	2.131	10	12.1	2.1
Shellfish	0.0226	0	3.8	3.8
	0.0226	0	4.0	4.0
	0.0226	2.5	6.4	3.9
	0.0226	5	9.1	4.1
	0.0226	10	14.1	4.1

Table II. Cadmium Contents in Individual Organs of Meretrix Lusoria (Shirahama Seaside, Himeji) Samples (ppm)

Organ	1		2		3		4		Av		Enrichment Factors
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	
Mantle	0.043	0.25	0.021	0.13	0.014	0.085	0.021	0.13	0.025±0.013	0.15±0.07	1,200
Gills	0.044	0.25	0.081	0.48	0.076	0.50	0.076	0.46	0.069±0.017	0.42±0.12	3,200
Siphon	0.013	0.076	0.017	0.090	0.012	0.067	0.011	0.066	0.013±0.003	0.075±0.011	580
Foot	0.010	0.039	0.022	0.085	0.015	0.058	0.015	0.058	0.016±0.005	0.060±0.019	460
Foot Muscle	0.031	0.12	0.054	0.21	0.035	0.15	0.031	0.11	0.038±0.011	0.15±0.05	1,200
Liver	0.067	0.28	0.094	0.42	0.092	0.40	0.057	0.23	0.078±0.018	0.33±0.09	2,500
Kidney	4.4	20	5.7	27	3.6	18	6.3	29	5.0±1.2	24±5	180,000
Organ of Keber	0.24	1.1	0.25	1.1	0.12	0.54	—	—	0.20±0.07	0.91±0.32	7,000
Labial Palps	0.040	0.22	0.051	0.26	0.020	0.11	0.027	0.11	0.035±0.014	0.18±0.08	1,400
Adductor Muscles	0.031	0.13	0.012	0.050	0.020	0.087	0.035	0.15	0.025±0.010	0.10±0.04	770

Table III. Cadmium Contents in Sea Water and Sediments (Shirahama Seaside, Himeji)

	Depth (m)	Cd in Sea Water (ppb)	Cd in Sediments (ppm)
Shirahama	I 0	0.14	0.13
Seaside	II 0	0.15	0.18
	III 0	0.11	0.13

Distribution of Cadmium

The results for the distribution of cadmium in individual organs of four shellfishes gathered at Shirahama seaside were summarized in Table II, and the data for the sea water and sedimentary material were shown in Table III. The enrichment factor for cadmium in the individual organs compared with the concentration in sea water was shown in the last column of Table II. As shown in Table II there was a comparatively narrow variation in the concentration of cadmium in the same organs of the four samples. Cadmium in shellfish was to a high degree enriched in kidney, considerably enriched in organ of Keber, gills and liver, and was relatively poor in shiphon, foot and adductor muscles. A similar result was obtained in the shellfish sample collected at Fukuura seaside, Ako city as shown in Table IV.

Table V showed cadmium content in shells which were cut up along the line of growth in order of the length from umbo. It was difficult to find the clear correlation between the cadmium concentration in shells and the length from umbo.

The apparent distribution coefficient of cadmium between the mantle and shell of clam can be calculated by means of the following equation:

$$D = C_{Ca} \cdot m_{CdCO_3} / C_{Cd} \cdot m_{CaCO_3} \quad (1)$$

where C_{Ca} and C_{Cd} are the total concentration of calcium and cadmium ions in sea water, and m_{CaCO_3} and m_{CdCO_3} are the mole fractions of calcium and cadmium in the shell of clam, respectively.

The concentrations of cadmium and calcium in whole shell were about 0.27 ppb and 39.4%, and the concentrations of these ions in the mantle were 25 ppb and 0.069%,

Table IV. Cadimium Contents in Individual Organs of Meretrix Lusoria (Fukuura Seaside, Ako, Hyogo)

	Wet (ppm)	Dry (ppm)		Wet (ppm)	Dry (ppm)
Mantle	0.021	0.11	Liver	0.035	0.14
Gills	0.068	0.39	Kidney	5.0	20
Siphon	0.015	0.085	Organ of Keber	0.45	1.9
Foot	0.012	0.045	Labial Palps	0.021	0.096
Foot Muscle	0.016	0.056	Adductor Muscles	0.022	0.084

Table V. Concentration of Cadmium in Shell of Clam (Shirahama Seaside, Hyogo)

Sample No.	Length from Umbo (mm)	Left Valve Cd (ppb)	Right Valve Cd (ppb)
1	0~23	0.29	0.65
	23~37	0.25	0.13
	37~49	0.41	0.20
	49~56	0.10	0.33
	56~62	0.17	0.10
	62~69	0.45	0.34
	69~75	0.12	0.25
	75~81	0.20	0.41
2	0~26	0.26	0.67
	26~38	0.16	0.36
	38~46	0.24	0.37
	46~51	0.20	0.44
	51~56	0.30	0.61
3	0~20	0.20	0.16
	20~33	0.10	0.10
	33~42	0.25	0.16
	42~49	0.15	0.27
	49~53	0.25	0.13

respectively. Therefore, the apparent distribution coefficient of cadmium was found to be about 1.9×10^{-5}, while true distribution coefficient of cadmium between the solution phase and the aragonite crystal which is the principal inorganic constituent of the shell was 44[7] and this is extremely larger than the value *in vivo*. Since the basicity of the cadmium ion for coordination is less than that of the divalent first transition metals, cadmium ion will probably not form coordinate links so tightly with organic ligands in shellfish such as peptides, but may be associated in some biological systems involving other forms of linkage.

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