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A Study of the Inter-elemental Effect in the Carbon-furnace Atomic-absorption Spectrometry of Selenium and Mercury in Biological Samples by the Palladium-addition Method

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In the electrically-heated carbon-furnace atomic-absorption spectrometry of selenium and mercury, inter-elemental interference is present between the two elements. Mercury has no effect on selenium sensitivity in a simple inorganic selenium solution. When, however, the solution contains organic materials such as 0.5% albumin, the selenium sensitivity is decreased. The presence of mercury in addition to the organic material further decreases the sensitivity to about a half of that of a solution containing only selenium and albumin. The addition of 0.94 mmol dm^{-3} palladium ion completely removes the negative interferences due to organic materials and mercury. On the other hand, in mercury determination the presence of organic materials also decreases the mercury sensitivity. The addition of palladium, in contrast to selenium determination, enhances the mercury sensitivity about 50 times, independent of whether or not the solution contains organic materials.

It is known that some dolphins accumulate mercury and selenium in their livers at concentrations as high as 1.27 mmol dm^{-3}.[1,2] The chemical forms and the roles of these elements in livers are, however, still unknown. The concentration of the elements range from as low as 0.01 mmol dm^{-3} to more than 1.27 mmol dm^{-3}. As for the concentrations of these elements, it has been reported that the mercury concentration increases with the increase in the selenium concentration. However, there is no definite conclusion as to the cause of the accumulation of the two elements. This accumulation phenomenon is especially interesting from the viewpoints of the biochemical role of selenium in mammals, the toxicological effect of mercury, and the biochemical relation between the two elements.[3,4] Although the importance of selenium as an essential trace element is widely recognized today, no intensive study seems to have been carried out concerning the chemical forms and functions of these elements in dolphin livers. This is probably a result of the lack of a highly sensitive analytical method for these elements. Recently, the present authors have reported that the addition of palladium greatly enhances the sensitivities of both selenium and mercury, while removing interference due to organic materials in electrically-heated carbon-furnace atomic-absorption spectrometry.[5,6] In the present paper, inter-elemental interference between the two elements and the effects of organic materials on the sensitivities of the two elements in the palladium-addition method were studied. Although interference due to organic materials was investigated in previous studies[5,6] for both elements when they exist independently, it was found in the present study that the interference is completely different when the two elements co-exist in a solution. The correct estimation of such interference is indispensable to an accurate analysis of such samples as dolphin livers.

As for the palladium-addition method for selenium determination, Taga *et al.*[7] and S. X.-Quan *et al.*[8] have reported that palladium slightly enhances the sensitivity. However, our results show that the effect is remarkable and by far larger than has been reported previously. Moreover, there has been no report concerning the inter-elemental interference between selenium and mercury.

Experimental

Equipment and Measurement Conditions. A Shimadzu AA-640-13 atomic-absorption spectrometer equipped with a Shimadzu GFA-4 graphite-furnace atomizer was used. A selenium wavelength of 196.0 nm and a mercury wavelength of 253.7 nm were used. The measurement conditions were as follows; Se: dry 150 °C, 40 s, ramp mode; ash 500 °C, 60 s, ramp mode; atomize 2100 °C, 6 s, step mode; Hg: dry 150 °C, 40 s, ramp mode; ash 400 °C, 60 s, ramp mode; atomize 2000 °C, 6 s, step mode.

Reagents and Samples. Standard solutions of mercury ($HgCl_2$), selenium (Na_2SeO_3), and palladium ($PdCl_2$) were prepared by diluting a commercial 1000 mg dm^{-3} solution obtained from the Wako Co. Bovine albumin and methionine and selenomethionine from the Sigma Co. were used.

An extract solution of dolphin livers was prepared by extracting a homogenate of the livers with a 0.2 M ammonium acetate buffer (pH 7). The solution was then submitted to ultrafiltration with a YM-2 filter, Amicon Co.; the filtrate (M. W. less than 1000) was used for analysis. When comparing selenium results for undecomposed and acid-decomposed extract solutions, the filtrate was decomposed by adding HNO_3, H_2O_2, and $HClO_4$.

Procedure. A palladium solution of 9.4 mmol dm^{-3} was added to a sample solution before measurement so that the solution contained palladium at 0.94 mmol dm^{-3}.

Thirty mm^3 of the solution was then injected into a carbon furnace with an Eppendorf micropipette.

Results and Discussion

Effect of Palladium Addition for Sensitivity Enhancements of Selenium and Mercury in Biological Samples. The effect of palladium addition on selenium sensitivity was investigated; the results are shown in Fig. 1(A). Palladium neither enhances nor decreases the sensitivity for selenium when selenium exists in a solution as inorganic selenite. When selenium is added to an extract solution of dolphin livers, however, the selenium sensitivity is reduced to less than a twentieth; this negative interference is due to organic materials in the solution. However, the sensitivity is completely recovered to that of a simple inorganic selenium solution when 0.94 mmol dm^{-3} of palladium is added to the extract solution. The effect is remarkable, and it should be noted that the intercept of the calibration curve for the Pd-added extract solution corresponds to the selenium originally contained in the solution. In other words, selenium in such biological samples can be determined by the standard addition method with the aid of palladium addition.

On the other hand, in the mercury determination shown in Fig. 1 (B), palladium addition enhances the sensitivity about 50 times, even in a simple inorganic solution. Mercury sensitivity in an extract solution is so much decreased that no signal is observed. However, with the addition of palladium, mercury sensitivity is recovered to about a half of that of a simple inorganic mercury solution with palladium added.

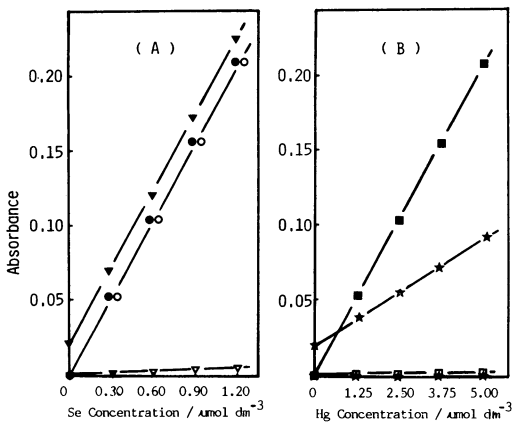

Fig. 1. Effect of palladium addition on selenium and mercury sensitivities.
(A) ○: An aqueous solution, ●: ○+Pd 0.94 mmol dm^{-3}, ▽: an extract solution of dolphin's livers, ▼: ▽+Pd 0.94 mmol dm^{-3}, (B) □: An aqueous solution, ■: □+Pd 0.94 mmol dm^{-3}, ☆: an extract solution of dolphin's livers, ★: ☆+Pd 0.94 mmol dm^{-3}.

Effect of Organic Materials on the Palladium-enhancement Effect. In order to study the effect of organic materials in the palladium-addition method, albumin was added to a selenium (1.27 μmol dm^{-3}) or mercury (9.97 μmol dm^{-3}) solution at various concentrations and the sensitivities were compared. Figure 2 clearly exhibits the effect. The existence of organic material, even at concentrations of less than 1%, drastically decreases the sensitivities of both selenium and mercury. The addition of palladium, however, completely restores the sensitivity to that of a solution without organic materials, even when albumin is present at concentrations of up to 2.0% during selenium determination. On the other hand, mercury sensitivity is increased by palladium addition at any albumin concentration, as is shown in Fig. 2.

Effect of Mercury in the Palladium-addition Method of Selenium. For the accurate determinations of mercury and selenium in extract solutions of dolphin's livers, inter-elemental effects between the two elements must be studied, especially with regard to the present palladium-addition method. The effect of palladium addition in the selenium analysis of a solution containing mercury is shown in Fig. 3. As Fig. 3 shows, the existence of mercury or the addition of palladium has no effect on the selenium sensitivity in a simple inorganic selenium solution. However, when the solution contains albumin (0.5%), the selenium sensitivity is decreased to about one-fifth of that of a simple inorganic selenium solution. The addition of palladium to a solution containing both selenium and organic materials restores the selenium sensitivity to that of a simple inorganic solution. Figure 3 also shows that the presence of mercury in addition to albumin further decreases the selenium sensitivity to about a half of that of a selenium solution containing only albumin.

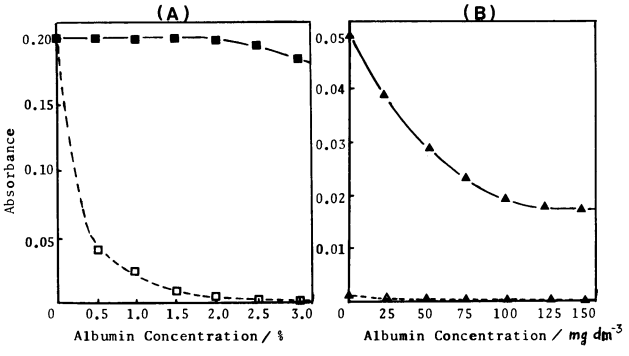

Fig. 2. Interference due to albumin in selenium and mercury determinations and effect of palladium addition for removal of the interference and enhancement of the sensitivity.
(A) Se 1.27 μmol dm^{-3}, □: without Pd, ■: with Pd (0.94 mmol dm^{-3}), (B) Hg 9.97 μmol dm^{-3}, △: without Pd, ▲: with Pd (0.94 mmol dm^{-3}).

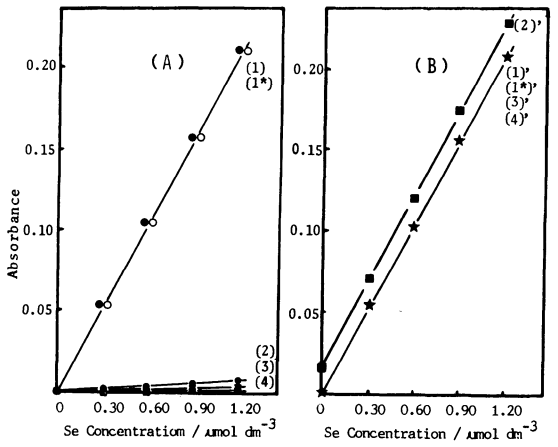

Fig. 3. Interferences of mercury and albumin on selenium determination and removal of the interferences by palladium addition.
(1): An aqueous selenium solution, (1*): (1)+Hg 1.25 μmol dm^{-3}, (2): an extract solution of dolphin's livers, (3): (1)+albumin 0.5%, (4): (3)+Hg 1.25 μmol dm^{-3}, (1)': (1)+Pd 0.94 mmol dm^{-3}, (1*)': (1*)+Pd 0.94 mmol dm^{-3}, (2)': (2)+Pd 0.94 mmol dm^{-3}, (3)': (3)+Pd 0.94 mmol dm^{-3}, (4)': (4)+Pd 0.94 mmol dm^{-3}.

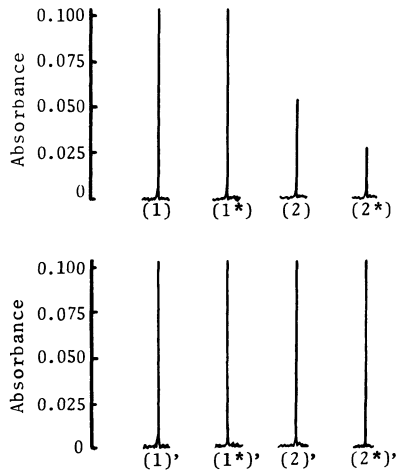

Fig. 4. Selenium sensitivity dependence on the chemical form and effect of palladium addition.
(1): Selenite (Se 0.63 μmol dm^{-3}), (1*): (1)+Hg 0.63 μmol dm^{-3}, (2): selenomethionine (Se 0.64 μmol dm^{-3}), (2*): (2)+Hg 0.63 μmol dm^{-3}, (1)': (1)+Pd 0.94 mmol dm^{-3}, (1*)': (1*)+Pd 0.94 mmol dm^{-3}, (2)': (2)+Pd 0.94 mmol dm^{-3}, (2*)': (2*)+Pd 0.94 mmol dm^{-3}.

The addition of palladium to such a solution completely restores the sensitivity. All the severe negative interferences due to organic materials and/or mercury can be completely removed by palladium addition, as is shown in (B) of Fig. 3.

In order to examine whether or not the selenium sensitivity is dependent on the chemical form, the sensitivities of selenite and selenomethionine solutions were compared. The effects of palladium addition were also compared for the two solutions; the results are shown in Fig. 4. It should be noted that the selenium sensitivity of selenomethionine is less than a half of that of a selenite solution. The presence of 0.63 μmol dm^{-3} of mercury does not affect the selenium sensitivity in a selenite solution. However, the sensitivity is decreased to nearly a half when selenium exists as selenomethionine. Considering the results of Figs. 3 and 4 together, it can be inferred that mercury decreases the selenium sensitivity only in organic-matrix solutions. "Organic matrix" means that either the solution contains organic substances in addition to inorganic selenium or selenium is contained as organic selenium molecules. The addition of palladium completely restores the sensitivity in any organic-matrix solution with or without mercury.

Effect of Selenium in the Palladium-addition Method of Mercury. It was found that selenium additionally enhances mercury sensitivity when added together with palladium. Figure 5 shows the dependence of the mercury sensitivity on the selenium concentration in a solution to which

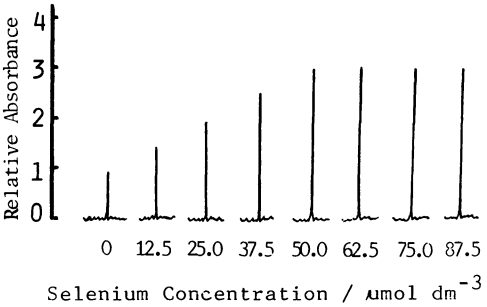

Fig. 5. Enhancement effect of selenium in palladium addition method of mercury. The solution contains Hg 0.05 mmol dm^{-3} and Pd 0.94 mmol dm^{-3}.

0.94 mmol dm^{-3} of palladium has already been added. The mercury sensitivity is linearly increased with an increase in the selenium concentration until it reaches 50 μmol dm^{-3}. At selenium concentrations higher than 50 μmol dm^{-3}, however, no more sensitivity enhancement is observed. It should be noted that 50 μmol dm^{-3} of selenium corresponds to a 1:1 molar ratio of Se and Hg; this suggests that the sensitivity enhancement may result from a 1:1 complex formation between Se and Hg, a complex which is retained in a carbon furnace up to higher temperatures than when only mercury is present. A similar enhancement effect for mercury by sulphur addition has been reported by Ediger.[9] The effect is believed to be due to the formation of mercury sulphide.

Effect of Palladium Addition for Mercury Determination in Solutions of Various Matrices. The effects

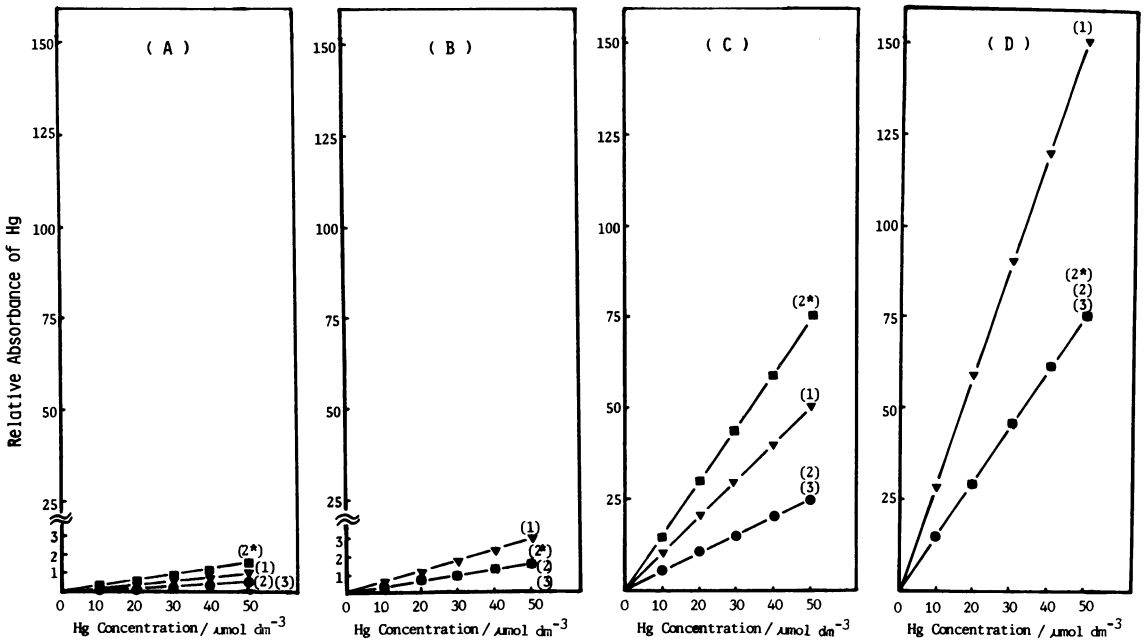

Fig. 6. Calibration curves for mercury in the presence or absence of selenium, organic materials and palladium. A (1): An aqueous solution of mercury (0.05 mmol dm^{-3}), (2): (1)+methionine 0.067 mmol dm^{-3}, (2*): (1)+selenomethionine 0.051 mmol dm^{-3}, (3): (1)+albumin 60 mg dm^{-3}. B (1): A(1)+selenite (Se 0.064 mmol dm^{-3}), (2): A(2)+selenite (Se 0.064 mmol dm^{-3}), (2*): same as A(2*), (3): A(3)+selenite (Se 0.064 mmol dm^{-3}). C (1): A(1)+Pd 0.94 mmol dm^{-3}, (2): A(2)+Pd 0.94 mmol dm^{-3}, (2*): A(2*)+Pd 0.94 mmol dm^{-3}, (3): A(3)+Pd 0.94 mmol dm^{-3}. D (1): A(1)+Se 0.064 mmol dm^{-3}+Pd 0.94 mmol dm^{-3}, (2): A(2)+Se 0.064 mmol dm^{-3}+Pd 0.94 mmol dm^{-3}, (2*): A(2*)+Pd 0.94 mmol dm^{-3}, (3): A(3)+Se 0.064 mmol dm^{-3}+Pd 0.94 mmol dm^{-3}.

TABLE 1. COMPARISON OF SELENIUM DETERMINATIONS FOR UNDECOMPOSED AND ACID-DECOMPOSED EXTRACT SOLUTIONS OF DOLPHIN LIVERS (mmol dm^{-3})

Sample[a)]	Undecomposed solution	Acid-decomposed solution
1	26.8	24.1
2	14.1	17.1
3	16.4	15.2
4	9.5	10.3

a) One g of liver was extracted with a 10 cm^3 solution. The Extract solutions were as follows: 1, 1 mol dm^{-3} phosphate buffer (pH 7); 2, 1 mol dm^{-3}, phosphate buffer (pH 7) +1% SDS; 3, 1% NaCl; 4, 1 mol dm^{-3} phosphate buffer (pH 7) +2% Triton.

of selenium compounds, organic materials, and palladium on the mercury sensitivity are summarized in Fig. 6. Figure 6 (A) shows that the mercury sensitivity is reduced to almost a half of that of a simple mercury solution when methionine or albumin is present. On the contrary, the presence of selenomethionine increases the sensitivity about 1.5 times. When inorganic selenium is added to the solution of (A) in Fig. 6, the sensitivity is increased about 3 times. The fact that the sensitivities of (2) and (3) in Fig. 6 (B) are almost equal to that of (2*) suggests that the enhancement effect of selenium is independent of its chemical form. Although selenium enhances the mercury sensitivity in organic-matrix solutions, it cannot completely remove the negative interference due to organic materials, as is shown in Fig. 6 (B). Figure 6 (C) shows the effect of palladium. The addition of palladium enhances the mercury sensitivity about 50 times. This enhancement ratio is independent of whether the solution contains organic materials or selenium. The addition of both selenium and palladium enhances the mercury sensitivity about 150 times. This enhancement ratio is equal to the sum of the enhancement ratios of the two elements. Table 1 compares the selenium determinations obtained for undecomposed and acid-decomposed extract solutions of dolphin livers. All the measurements were done by means of the palladium-addition method; the results show that the present palladium-addition method makes possible the direct determination of selenium in an undecomposed solution containing organic materials.

The inter-elemental effects of selenium and mercury may be summarized as follows. Mercury decreases the selenium sensitivity, whereas selenium enhances the mercury sensitivity. The presence of organic materials decreases the sensitivities for both elements. The addition of palladium to a selenium solution

with an organic matrix completely removes the negative interferences caused by organic materials and mercury, whereas the addition of palladium enhances the mercury sensitivity in solutions with both inorganic and organic matrices.

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