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## **II. 4. Determination of Trace Metals in Biological Samples by PIXE Analysis After Solvent Extraction and Polystyrene-Film Collection**

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Particle-Induced X-ray Emission analysis (PIXE analysis) is a novel technique for elemental analysis and it has high sensitivity for first transition metals. But determination of first transition metals in biological samples is not easy because these metals are involved at trace level as often under  $\mu\text{g/g}$  level. Therefore separation and concentration of these metals previous to PIXE analysis is necessary for the reliable determination. In PIXE analysis, a X-ray spectrometry cannot be often performed by high back ground X-ray. This back ground X-ray is mainly generated by the bremsstrahlung of charged particles and incident secondary electrons. So a preparation technique of a thinner target ( $<1\text{mg/cm}^2$ ) after concentration of first transition metals is necessary to perform the high sensitivity of PIXE analysis. In this paper, we developed the selective and quantitative separation of several first transition metals, vanadium, manganese, iron, cobalt, nickel, copper and zinc in biological samples by solvent extraction with diethyldithiocarbamate (DDTC), and the collection of these extracted metals into a thin polystyrene film. The present technique is applied to NBS SRM 1572 Citrus Leaves and marine macro-alga samples.

Alga samples, *Undaria pinnatifida* (Wakame) were obtained from Oskika pen, Miyagi, Japan, in July 1989. After collection they were subject to the lyophilization for 48h and powdered. One gram of biological sample was digested by nitric acid and then evaporated to dryness. It was dissolved in acetate buffer solution and vanadium, iron, cobalt, nickel, copper and zinc in the solution were extracted with 0.01M DDTC into benzene at pH 3.5 -5 by shaking. The organic extract was taken out. Manganese in the remaining aqueous solution was extracted at pH 5 - 7.5 with another benzene.

The total extract obtained by the duplicate extraction was added 10mg of polystyrene and was added tris (2-thenoiltrifluoroacetate)scandium (III) as an internal standard for PIXE analysis. The benzene solution was subject to the lyophilization for 10h to remove benzene and then the metal-DDTC complexes were concentrated into the resulting polystyrene and it was dissolved again in small potion of benzene. The benzene solution was spread on the Mylar film and dried. The polystyrene film ( $1\text{mg/cm}^2$ ) was obtained by

peeling from the Mylar film. To examine a stability of the polystyrene film for charged particle bombardment, it was irradiated with 3 MeV proton beam (50 nA) for 90min. However, no decomposition or melting of the film was observed.

Recovery of trace metals were measured by  $\gamma$ -activity of acid digested solution of alga sample labeled with  $^{48}\text{V}$ ,  $^{54}\text{Mn}$ ,  $^{59}\text{Fe}$ ,  $^{57}\text{Ni}$ ,  $^{57}\text{Co}$ ,  $^{67}\text{Cu}$  and  $^{65}\text{Zn}$ .  $^{48}\text{V}$  and  $^{65}\text{Zn}$  were produced by 18 MeV protons from a cyclotron of Tohoku University, and  $^{57}\text{Ni}$  and  $^{67}\text{Cu}$  were produced by 50 MeV bremsstrahlung from an LINAC of Tohoku University. Percentage of recovery of solvent extraction for each metal was more than 99.5%<sup>1)</sup> and recovery of concentration into polystyrene were more than 97%. In the present method, the several trace metals in the 1g of biological sample were separated and concentrated into the 10mg of 1mg/cm<sup>2</sup> polystyrene film target without any loss.

The sample and the comparative standard were irradiated by 3 MeV proton beam (Beam current, 50nA; Beam diameter, 3mm) from the cyclotron of Tohoku University for 20min. X-ray spectra were measured with a Si (Li) semiconductor detector connected with 4096 channel pulse height analyzer. X-ray spectrometry were carried out by the least squares fitting computer program. Each element was determined by  $K_{\alpha}$  X-ray peak area.

Contamination from reagents and/or environments is serious problem in trace analysis. To determine the contamination of this method, the purity of the polystyrene film made by the present separation and concentration procedure without biological sample was subjected to PIXE analysis. Figure 1 shows the X-ray spectrum of the polystyrene film. There is no K X-ray peak except for sulfur and scandium from internal standard reagent. Thus the contamination of the present procedure is to be negligible.

Figure 2 shows the X-ray spectrum of a polystyrene film target obtained by the present method for the alga sample, *Undaria pinnatifida*. Application of PIXE analysis for first transition metals in marine macro-algae was not reported. K X-ray peaks of trace metals from the alga sample, vanadium, manganese, iron, nickel, copper and zinc are clearly observed, and there is almost no X-ray peak from alkali metals, alkaline earth metals and halogens contained at high levels in the alga sample. Therefore, X-ray spectrometry could be performed with high accuracy. Analytical results for a series of first transition metals in NBS SRM 1572 Citrus Leaves and the alga sample are summarized in Table 1. Four trace metals, manganese, iron, copper and zinc in the Citrus Leaves sample could be determined with high accuracy and precision. For example, copper was found to  $16.2 \pm 0.1 \mu\text{g/g}$  ( $n = 3$ ), and this result was good agreement with NBS certified value ( $16.5 \pm 1.0 \mu\text{g/g}$ ). Six trace metals in the *Undaria pinnatifida* sample could be determined simultaneously. Nickel and copper at sub- $\mu\text{g/g}$  level could be determined and it shows this analytical method was sensitive.

This present method is able to perform the selective and quantitative separation and the high enrichment for trace element in biological samples and is able to prepare the thin film

target to be suitable for PIXE analysis. This method can be applied to the simultaneous determination of trace amount of first transition metals in other biological samples.

## Reference

- 1) N. Suzuki, Y. Iwata and H. Imura, Intern. J. Environ. Anal. Chem. 30 (1987) 289.

Table 1. Trace metal analysis of NBS SRM 1572 Citrus Leaves and an alga sample (*Undaria pinnatifida*) by the solvent extraction/PIXE analysis

Metal	Citrus Leaves		Certified Value/ $\mu\text{g}\cdot\text{g}^{-1}$	Alga sample	
	This work / $\mu\text{g}\cdot\text{g}^{-1}$	(n)		This work / $\mu\text{g}\cdot\text{g}^{-1}$	(n)
V	-		-	$1.4 \pm 0.15$	(3)
Mn	$21 \pm 2$	(3)	$23 \pm 2$	$8.5 \pm 0.54$	(3)
Fe	$88 \pm 9$	(3)	$90 \pm 10$	$41 \pm 6.7$	(3)
Ni	-		$0.6 \pm 0.3$	$0.52 \pm 0.10$	(3)
Cu	$16.2 \pm 0.1$	(3)	$16.5 \pm 1.0$	$0.54 \pm 0.09$	(3)
Zn	$27.6 \pm 0.2$	(3)	$29 \pm 2$	$18.8 \pm 0.2$	(3)

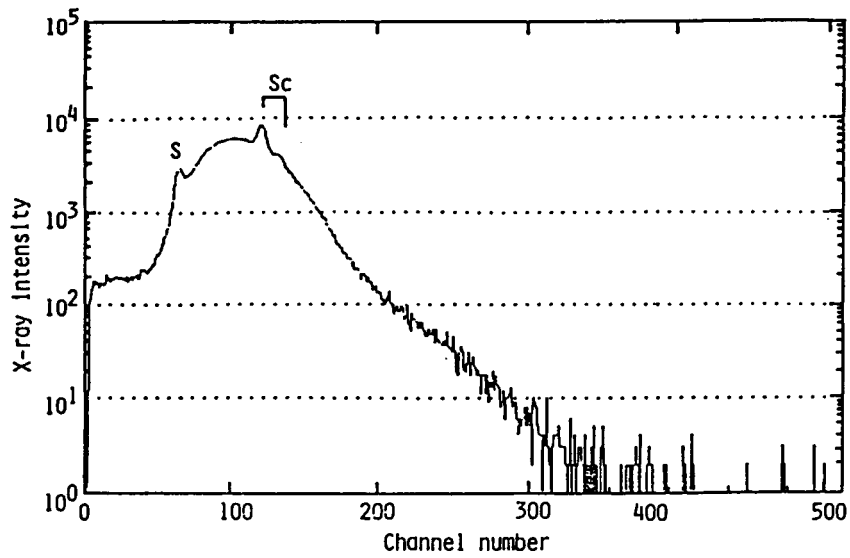


Fig. 1. X-ray spectrum of the polystyrene film obtained by the blank test for the preseparation and preconcentration method.

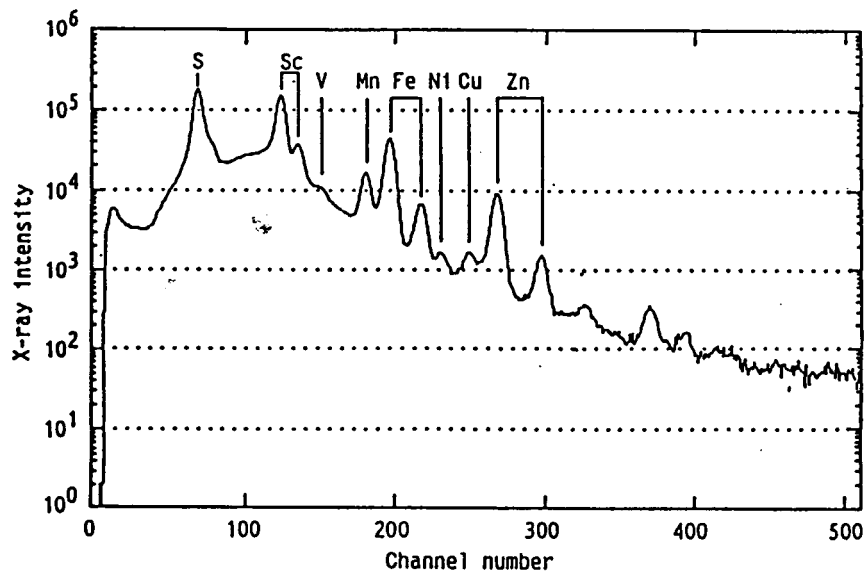


Fig. 2. X-ray spectrum of the polystyrene film target containing trace metals separated from *Undaria pinnatifida* (Wakame) sample.