Trace elemental analyses of beverages and biological materials by TXRF spectrometry

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1. Introduction

Total reflection of X-rays occurs on the surface of a measured object by X-ray irradiation at an extremely low glancing angle (less than 0.1°). X-rays penetrate into a measuring object to a depth of only several (≈ 10) nm. Therefore, elements on the surface of the sample carrier are excited efficiently and the background intensity occurring from the sample carrier is extremely low. As a result, spectra with high S/N ratio can be obtained. High-sensitivity analysis for trace elements on the surface of the sample carrier can be performed by the total reflection method with a total reflection X-ray fluorescence (TXRF) spectrometer. Concentrations of μg/L (ppb) for inorganic components in a water sample are easily determined by using the internal standard method. Analysis results of beverage samples (drinking water and soft drink), milk, coffee, tea and wine by TXRF are reported[1], [2]. Many more TXRF results of serum samples by acid decomposition are also reported[3]. Recently, TXRF spectrometry is widely used for liquid analysis because TXRF spectrometry was made an official liquid analysis method by the International Organization for Standardization (ISO). In this paper, water analysis by TXRF is described and, beverages and serum are introduced as analysis examples.

2. Analysis of water samples

In water analysis by TXRF spectrometry, a 10μL water sample was pipetted onto a quartz glass carrier, and its dried residue was analyzed. Pictures of dried residues of liquid samples on quartz glass carriers for distilled water, tea, wine, and salt water are shown in Fig. 1 on a scale of inorganic component concentration. The dried residue spot sizes of tap water and beverage were about 1 mm. Low background intensities on TXRF spectrum were obtained although tap water and beverage contain several 100 mg/L (ppm) of inorganic components. The dried residues of soft drinks and alcoholic drinks are larger compared to tap water and drinking water because they contain inorganic components such as minerals and salt as well as organic components including sugar and alcohol. The background in TXRF spectrometry becomes higher as a function of the components and volume of dried residue. Therefore, in this case, lower limits of detection (LLDs) of analytical elements show larger values. The heterogeneity of dried residues causes worsening of the reproducibility of analytical values due to analytical errors by the absorption
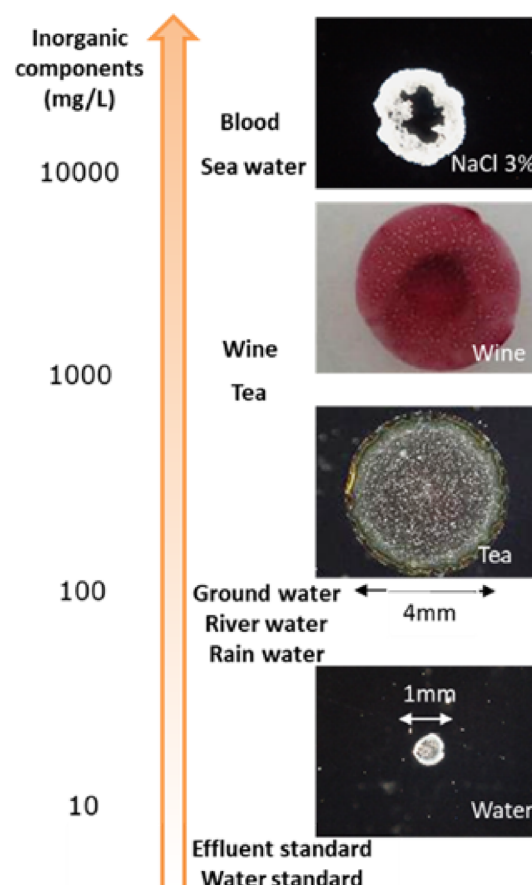
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Fig. 1. Pictures of dried residues for liquid sample on quartz glass carriers.

effect. Therefore, dilution, acid decomposition and desalinization treatments might be necessary according to the sample type and analytical requirements.

3. Quantitative analysis by the internal standard method

In the TXRF spectrometry, the internal standard method is generally used for quantitative analysis because fluorescent X-ray intensities are unstable because they are affected by the shape and the absorption by components within the dried residue and distance variations to the detector. In the internal standard method, an internal standard solution with known concentration is added to an analytical sample and the fluorescent X-ray intensities of an analytical elements are corrected by the fluorescent X-ray intensity ratios of the analytical and internal standard elements.

The concentration C_i' of an analytical element i in the mixture solution of the analytical sample and the internal standard solution is calculated by the following equation:

$$C_i' = \frac{C_{IS}' \times I_i \times k_{IS}}{I_{IS} \times k_i}$$

The relative sensitivity coefficient k is sensitivity difference among elements. They are obtained as a result of the theoretical calculation[5] using physical constants of analytical lines, optical system of the spectrometer and the experimental condition. The concentration of the analytical element in the mixture solution is obtained from the known concentration of internal standard element, intensities, and the relative sensitivity coefficients of the internal standard and the analytical elements. The concentration C_i of the analytical element before adding the internal standard solution is calculated by using individual volumes in sample preparation.

$$C_i = C_i' \times \frac{V_i + V_{IS}}{V_i}$$

C_i' : Concentration of the analytical element in mixture solution
C_{IS}': Concentration of an internal standard element in mixture solution
C_i : Concentration of an analytical element in sample
C_{IS}: Concentration of internal standard element in internal standard solution
I_i : Fluorescent X-ray intensity of analytical element
I_{IS} : Fluorescent X-ray intensity of internal standard element
V_i : Volume of analytical sample
V_{IS}: Volume of internal standard solution
k_i : Relative sensitivity coefficient of analytical element
k_{IS} : Relative sensitivity coefficient of internal standard element

The criteria to select the internal standard element are that the element is not natively present in the sample and that there is no spectral overlap with analytical elements. Elements such as Sc, V, Ga, and Y are good candidates. Gallium is typically used as an internal standard element for environmental water and beverage samples. The internal standard solution is added to the sample in roughly comparable concentration to the analytical element. Also, the concentration of the internal standard element needs to be prepared such that a good repeatability (counting statistics) of fluorescent X-ray intensities can be obtained. For example, when a component with mg/L concentration in beverage sample is analyzed, a 10 μL of a 100 mg/L standard solution (for the ICP or AAS) is added to 990 μL sample. (Concentration of the internal standard is 1 mg/L in the mixture solution.)

4. Apparatus

NANOHUNTER II, Rigaku's bench-top total reflection X-ray fluorescence spectrometer, was used for the experiment. The excitation source consists of a fine focus 600 W Mo target X-ray tube run at 50 kV and 12 mA. It is equipped with a large detection area (50 mm^2) silicon drift detector (SDD) to achieve high sensitivity. A double-stacked synthetic multilayer mirror reflects X-rays of two excitation X-ray energies according to Bragg's law ($n\lambda$=2d sinθ) of X-ray diffraction phenomena: X-rays of 17.4 keV from the characteristic X-rays of Mo Kα and high energy X-rays of 30 keV from (continuous) Bremsstrahlung X-rays. NANOHUNTER II enables to analyze an element range; ^{13}Al to ^{92}U. Incident angle of 0.025° and measurement time of 1000 seconds were used.

5. Analysis of trace elements in fresh water certified reference material

NIST 1643f fresh water certified reference material (trace elements in fresh water) was analyzed. Analytical values and LLDs of analytical elements were evaluated. Ten μL Ga internal standard solution with 10 mg/L was
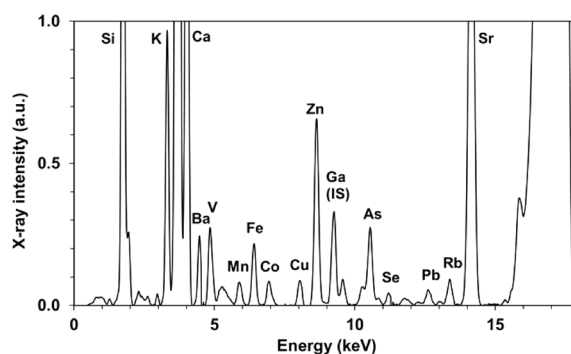

Fig. 2. TXRF spectrum of NIST 1643f (Trace elements in fresh water).

Table 1. Analytical result of NIST 1643f (Trace elements in fresh water).

(Unit: μg/L)

Element	Certified value	Analytical value	Standard deviation*	LLD**
K	1913	2081	58	18.4
Ca	29140	30902	1292	13.9
Cr	18	13.7	1.8	4.4
Mn	37	47.8	2.4	1.8
Fe	93	101	4.2	1.4
Co	25	24.5	0.5	1.3
Cu	21	22.3	2.9	0.96
Zn	74	74.7	5.6	0.90
As	57	60.7	1.2	0.63
Se	12	11.5	0.8	0.73
Rb	13	14.3	0.5	0.82
Sr	311	380	9.6	0.84
Pb	18	17.7	1.6	2.5

* Standard deviation, n=5.

** LLDs were calculated by the following equation.

added to 990 μL of the sample. 10 μL of mixture solution was pipetted on quartz glass carrier and dried under reduced pressure. Quartz glass carriers were coated by silicone before sample preparation. Five samples were prepared on quartz glass carriers for evaluating the reproducibility.

The TXRF spectrum of NIST 1643f fresh water certified reference material is shown in Fig. 2. The fluorescent X-ray peak of Si originates from the quartz glass substrate and the Ga peak is from the internal standard solution. Analytical results using the internal standard method with Ga are shown in Table 1. The analytical results showed good agreement with the certified values of NIST 1643f certified reference material. Coefficients of variation with five replicates are 2 to 13%. The LLDs of the analyte elements were calculated by 3 times the standard deviation of background intensities. LLDs of transition metals showed very low values varying from 0.6 to 1 μg/L.

$$\text{LLD} = 3\frac{C}{I_N}\sigma_B = 3\frac{C}{I_N}\sqrt{\frac{I_B}{t}}$$

C : Concentration of analytical element
I_N: Net intensity of analytical element
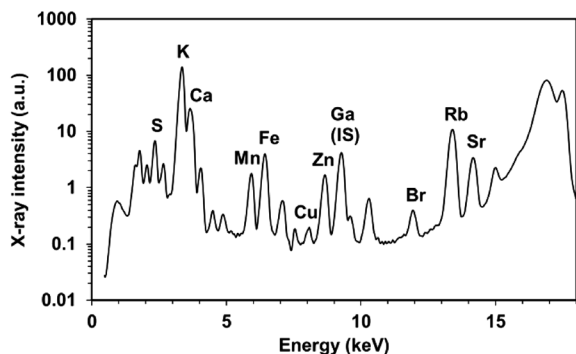I_B: Background intensity of analytical element
t : Measurement time

Fig. 3. TXRF spectrum of the Chilean red wine.

Table 2. Analytical result of Chilean red wine.
(Unit: mg/L)

Element	Analytical value	Reference (1)[6]	Reference (2)[7]
S	168	20−45	
K	803	250−880	254−757
Ca	66.7	41−100	41−101
Mn	1.86	0.5−1.4	0.5−0.9
Fe	3.25	0.3−4.2	0.3−3.8
Cu	0.05	0.02−0.37	0.02−0.37
Zn	0.72	0.1−1.4	0.1−1.4
Br	0.15	0.05−1.2	0.05−1.19
Rb	4.09	0.9−16	0.9−3.4
Sr	1.31	0.3−1.2	0.3−1.2

6. Analysis of wine

Wine contains trace elements of K, Ca, Fe, Mn, and Cu. Component analysis of wine is important for the viewpoints of dietary intake of trace metal elements and for identification to prevent locality and brand camouflage. TXRF has an advantage compared to other instrumental techniques due to its simple analysis method without decomposition and removal of alcohol, which would be needed in common analysis techniques such as ICP and AAS. A TXRF spectrum of a Chilean red wine sample is shown in Fig. 3. The errors of analytical values were decreased by diluting the wine sample with distilled water (sample to distilled water: 1 to 1) reducing matrix effects from light elements such as K and Ca[6]. Ten μL of Ga internal standard solution of 100 mg/L solution was added to 990 μL of the diluted red wine sample. The mixed sample was pipetted onto a quartz glass carrier (coated with silicone) and dried under reduced pressure. The TXRF analytical results and the reference values of the wine are shown in Table 2. The analytical values in the Chilean red wine are in the range of general reference values of elements in wine. Constituent concentrations are highly correlated with a wine's cited locality.

7. Analysis of As, Pb and Cd in leachate from tea

Soil contamination is a general concern in tea leaves. Therefore, an analysis of harmful components in leachate from tea leaves is studied. The focus in this
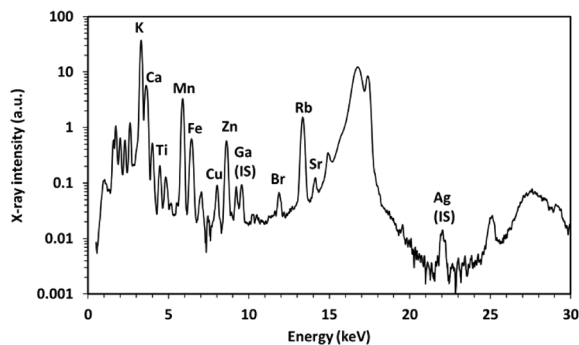

Fig. 4. TXRF spectrum of the leachate from tea leaves.

Table 3. Analytical value of the leachate from tea leaves
(Unit: mg/L).

Element	Analytical value
K	382
Ca	23.9
Ti	0.93
Mn	8.36
Fe	0.67
Cu	0.10
Zn	0.62
Rb	1.33

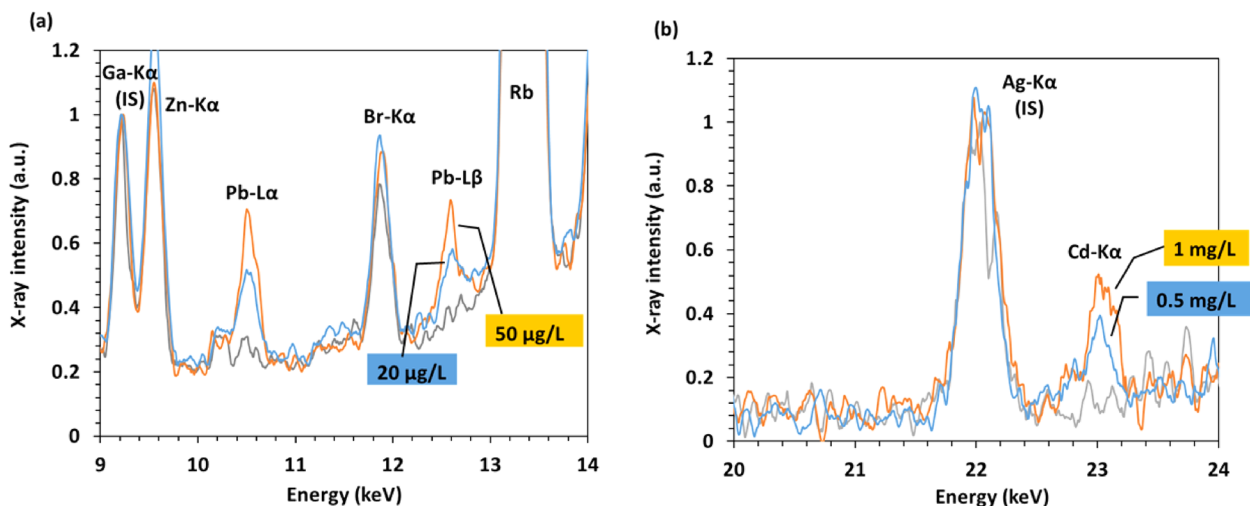**Fig. 5.** Pb and Cd spectra of the leachate from tea leaves.
(a) Pb standard solution added to 20 and 50 μg/L,
(b) Cd standard solution added to 0.5, and 1 mg/L.

report is analysis of harmful elements As, Pb and Cd in the leachate. The TXRF spectrum of the leachate from a tea leave samples boiled at 95°C for 10 min is shown in Fig. 4. Ten μL of 5 mg/L of Ga solution and 20 μL of 100 mg/L of Ag solution were added as internal standard solutions to 970 μL of the leachate. Gallium was used for the analysis of the elements such as As and Pb, whose X-ray energies are 18 keV. Silver was used for the analysis of the element such as Cd, whose X-ray energies are above 18 keV of high energy range. Ten μL of sample was pipetted onto a quartz glass carrier and dried under reduced pressure. The analysis results of the leachate are shown in Table 3. Several hundreds mg/L of K and within a range of sub mg/L to several mg/L of Ti, Mn, Fe, Zn and Rb were detected as inorganic components. Arsenic, Pb and Cd, as the focused harmful elements, were not detected. The leachate was analyzed by ICP-MS and the analyzed values of As and Pb were 1.0 and 0.6 μg/L respectively. The concentration of the elements seemed to be below detection limits of the TXRF measurement.

Figure 5 shows the spectra obtained by the leachate samples to which Pb and Cd standard solution were added. Resulting concentrations by adding were 20 and 50 for Pb, 0.5 and 1.0 mg/L for Cd. As shown, the peak intensities of the elements were increased by adding Pb and Cd. The LLDs for As, Pb and Cd were calculated to be 1.7, 6.4 and 112 μg/L respectively.

8. Analysis of serum sample

Serum samples, which contain high levels of organic components, such as protein in biological samples, should be prepared by acid decomposition and dilution. This procedure can decrease the amount of residue in the sample solution thus improving S/N of the TXRF spectrum (see Fig. 6). The decomposed serum sample was diluted 10 times with distilled water. Ten μL of 10 mg/L Ga internal standard solution was added to 990 μL of the diluted sample. Ten μL of mixture solution
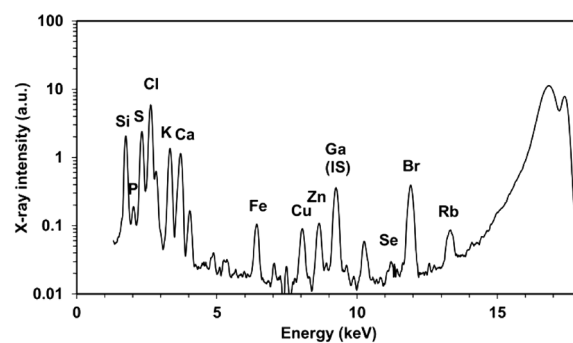

Fig. 6. TXRF spectrum of the blood serum.

Table 4. Analytical value of a serum sample and comparison with the analytical result of ICP-MS.

(Unit: mg/L).

Element	TXRF	ICP-MS
P	127	120
S	1016	1128
K	161	170
Ca	96.7	93.7
Fe	2.14	1.27
Cu	1.15	1.02
Zn	1.26	0.875
Se	0.140	0.136
Rb	0.512	0.165

was pipetted onto a quartz glass carrier and was dried under reduced pressure. The analytical results by TXRF are shown in Table 4 and compared with the analytical result by ICP-MS. Phosphorus, S, K and Ca as major components and Fe, Cu, Zn, Se and Rb as minor components were detected. The values showed good agreement with those from ICP-MS.

9. Conclusion

Analysis results of water and beverages by using benchtop total reflection X-ray fluorescence spectrometer, NANOHUNTER II were demonstrated. Liquid samples can be easily and rapidly analyzed by only pipetting and drying on a sample carrier. The sample preparation is easy relative to ICP-MS. Analytical elements were determined by using the internal standard method without preparing calibration curves. TXRF can be effectively applied to component analysis and screening analysis of various liquid samples.

References

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