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Spectrochimica Acta Part B 56 (2001) 2057–2062

SPECTROCHIMICA ACTA PART B

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Beijing synchrotron radiation total-reflection X-ray fluorescence analysis facility and its applications on trace element study of cells ☆

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Received 5 December 2000; accepted 30 June 2001

Abstract

The feasibility of total-reflection X-ray fluorescence (TXRF) analysis excited by synchrotron radiation applied to trace element analysis of biological cells is investigated. The Beijing synchrotron radiation TXRF facility and the related experimental method are also described. The elemental minimum detection limits of some standard reference materials are determined. The elemental compositions of a cluster of small intestine cells of a small white mouse are given, and hence the average trace element contents of the single small intestine cell are also obtained. With this technique, the changes of some trace elements in the cells of lung and cervix cancer before and after apoptosis are also preliminarily studied.

Keywords: Synchrotron radiation; Total-reflection X-ray fluorescence; Trace element; Cell

☆ This paper was presented at the 8th Conference on Total Reflection X-Ray Fluorescence Analysis and Related Methods, Vienna, Austria, September 2000, and is published in the Special Issue of *Spectrochimica Acta Part B*, dedicated to that conference.

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0584-8547/01/$ - see front matter
PII: S0584-8547(01)00283-X

1. Introduction

As we know total-reflection X-ray fluorescence (TXRF) analysis was developed based on an energy dispersion XRF technique [1,2]. The critical point is that the measured specimens were made into a solution, and a tiny droplet of the solution was then dropped on a polished surface of a silicon wafer or quartz crystal. By adjusting the incident angle of reflector (silicon wafer or quartz crystal) to the X-ray, the primary X-ray can be totally reflected by the reflector. As the droplet of the solution on the surface of the reflector became a very thin liquid drop spot after drying, the self-absorption effect of X-ray fluorescence can be neglected. Since the grazing incident angle was very small for TXRF, the XRF was excited effectively because of the long path of primary X-ray through the specimens, and at the same time the X-ray scattering background from the reflector could be greatly minimized. Hence TXRF becomes a more sensitive tool for trace element analysis than conventional XRF. Furthermore, the combination of TXRF with synchrotron radiation's excellent characteristics, such as collimation, polarization and high intensity, makes it an ideal analytical tool for trace elements [3,4]. Since this technique also has multi-element analysis capability and is a non-destructive analytical tool, it is very appropriate for the study of biomedical specimens.

Some essential trace elements in biological cells play an important role. Study of the compositions and their changes of the trace elements in cells can provide some valuable references for biomedicine science. The trace element contents within cells may vary to some extent under different physical and chemical conditions or various physiological and pathological statuses, such as irradiation, lack of oxygen, injury of free radical, cancer disease, etc. However, biological cell specimens usually contain very low elemental contents and are very difficult to detect by conventional XRF techniques. Several years ago using the conventional synchrotron radiation XRF (SR-XRF) technique, the trace elements in biological cells and their changes because of injury of free radical were investigated at our laboratory [5,6]. However, the sensitivities were not satisfactory, and also it usually took a very long measuring time to collect a spectrum. Since then the SR-TXRF technique was developed to analyze trace elements in biological cells in our group.

2. Experimental set-up and method

2.1. Instrumentation

The X-ray light sources in our experiments come from the 4W1A and 3W1A wiggler beam lines at Beijing Synchrotron Radiation Facility (BSRF) [7]. These beam lines can only provide X-ray white light (continuous spectrum), where the energy ranges are both from 3.5 to 35 keV. This energy range is suitable for TXRF analysis of most common trace elements. The electron energy in the storage ring is 2.2 GeV, with a current range of 25–120 mA, the brightness of the X-ray spectrum of 4W1A and 3W1A can reach approximately 10^{11} and 10^{12} ph/s per mrad^2 per mA per 0.1% BW, respectively. The SR-TXRF experimental facility is 43 m and 25 m long from the point of the light source for 4W1A and 3W1A, respectively. Since the emission angles of the light in horizontal and vertical directions are 0.3 and 0.1 mrad as well as 1.0 and 1.0 mrad for 4W1A and 3W1A beam lines respectively, this high collimation confers excellent characteristics to the SR-TXRF. As X-rays produced by synchrotron radiation are polarized within the plane of the electron orbit in the storage ring, the Compton and Rayleigh scattering light background in specimens can be minimized, and the elemental detection limits can be greatly improved [8].

The experimental station set-up mainly consists of two double knife slits, two ion chambers, a four-dimension specimen scanning stage system, a Si (Li) solid detection system, a laser collimation system, and an optical microscope [9]. The sketch diagram of SR-TXRF apparatus at BSRF is shown in Fig. 1. The X-ray beam spot was generated by using horizontal and vertical double knife slits. The spot sizes used in our experiments are approximately 85–500 μm (high) × 200–400 μm (wide), which are dependent upon the beam line

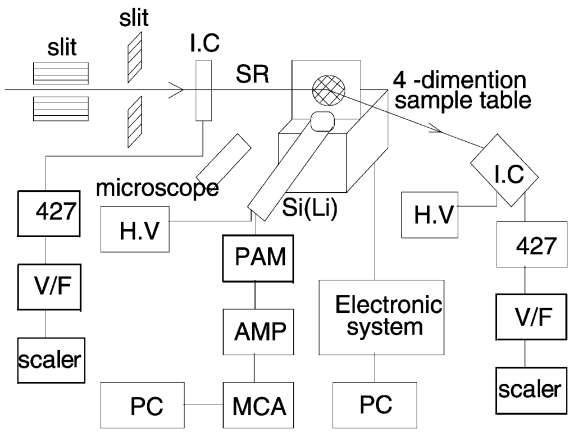

Fig. 1. Sketch diagram of the SR-TXRF apparatus at BSRF.

used and specimens. Since no monochromators were installed on the two beam lines at the present time, the synchrotron radiation white light was so intense that it is impossible to use an X-ray spot as large as the cell specimen spot. Otherwise the detector cannot work normally in this condition. The ion chamber in front of the specimen is used to observe the change of X-ray intensity. The second ion chamber monitors the relative position between the beam and the polished surface of the reflector. The energy dispersion detection system (Model Link ISIS) is made by Oxford Instrument Corporation, and its energy resolution is 134 eV (5.9 keV). The scanning stage includes three-dimension translations with 5 μm/step and one rotation with 0.0025°/step. An optical microscope was used to view the specimen exposing point.

2.2. Experimental method

The preparations of cell specimens for SR-TXRF experiments are given bellow. At first approximately 0.2–2.0 μl diluted standard specimen or cell solution was dropped on a polished surface of a silicon wafer, of surface roughness approximately 10 Å. After drying in a super clean air environment, a very thin dried liquid drop residue was formed on the polished surface of a silicon wafer with diameter of approximately 2.0–4.5 mm. Since the thickness of the dried cell liquid drop spot was so thin that it can be neglected with regard to self-absorption of fluorescence X-rays. Before analysis, the liquid cell drop without cell overlap was selected out and the number of the cells within the cell drop was counted using a microscope. The silicon wafer containing cell specimens was vertically put on a homemade specimen holder, which was then fixed on the four-dimension scanning stage.

In the SR-TXRF experiments, the incident X-ray beam was first aligned to set the beam parallel to the surface of the silicon wafer. Then the X-ray with a half of the incident beam width was allowed to pass through the polished surface of the silicon wafer by moving the specimen holder position. In the third step, the silicon wafer was turned into the state of TXRF, the spectrum of TXRF fluorescence can be seen. By changing the incident angle of TXRF, the same energy upper limit position of X-ray total reflection can be obtained for all unknown specimens and standard reference materials, i.e. the cut-off energy point, which was usually 20 keV in our case. Therefore the same excitation efficiency and the same ratio of elemental peak and background can be obtained. In the latter, the specimen spot was scanned continuously from top to bottom by using the selected X-ray spot with one by one steps. The live time for every scanning step was approximately 2–100 s. A complete SR-TXRF spectrum can be finally accumulated in this way for the measured specimen spot. The spectrum was normalized to the intensity of synchrotron radiation and total live time for every cell spot and standard specimen spot. For thin specimen spots, the trace element contents are proportional to the intensities of fluorescence X-ray. By comparing with the data of the standard reference specimens, the contents of trace elements in measured cell specimens can be obtained. The average elemental contents of a single cell can be also calculated by dividing the number of the cells in the cell specimen spot. At the same time, the elemental minimum detection limits (MDLs) can be determined by using the standard reference specimens.

Table 1
MDLs of standard reference materials of water and peach leaf determined by SR-TXRF at BSRF (pg)

Specimens	Cr	Mn	Fe	Ni	Cu	Zn	As	Sr	Pb
Water	4.4	–	–	4.2	4.2	2.2	–	–	4.8
Peach leaf	–	11.2	8.8	–	12.0	3.6	1.6	20.0	–

Table 2
The elemental contents of a cluster of small intestine cells (70 cells/0.5 μl) of a small white mouse and the average elemental contents of a single cell (pg)

Element	K	Ca	Cr	Mn	Fe	Cu	Zn
Cell cluster	26 750	1100	16	420	3730	70	210
Single cell	380	16	0.24	6	53	1	3

3. Experimental results and discussion

The MDLs of standard reference materials of water and peach leaf were determined by using the SR-TXRF technique. $MDL = 3C_i N_b^{1/2}/N_{net}$, where C_i is the concentration of the element i in the specimen, N_b is number of background counts, N_{net} is the net number of peak counts. For the standard specimen, the C_i is known, and N_b and N_{Net} could be calculated from the spectrum by AXIL software. In our case, the volumes used for the standard water and peach leaf solutions were both 2 μl. Therefore we can obtain the absolute detection limits by concentrations vs. sample volume. The data are shown in Table 1. For the common elements iron, copper and zinc, a few picograms MDLs can be determined.

With this new method, the trace element contents in a cluster of small intestine cells (70 cells/0.5 μl) of a small white mouse were measured and also the average elemental contents of a single cell were calculated. In this measurement, the standard reference material of pork liver was used, but since there is no data for the element chromium in pork liver standard, the element chromium was calculated by using standard reference material of water. The quantification procedure to calculate the elemental contents of cell specimens from standards is the same as the experimental method stated above. In our case, we think the sample can be regarded as

Table 3
The average relative elemental contents of a single cell of lung cancer determined by SR-TXRF (unit: counts of peak area) (47 and 72 cells in normal cancer and apoptosis cancer cells)

Element	Cancer cell before apoptosis	Cancer cell after apoptosis
Ti	0.85 ± 0.37	2.03 ± 0.64
V	0.55 ± 0.10	43.20 ± 1.57
Fe	512.80 ± 5.76	479.60 ± 4.22
Cu	5.46 ± 1.70	2.15 ± 0.55
Zn	32.10 ± 1.71	8.70 ± 1.11
As	1.72 ± 0.86	6.35 ± 1.25
Br	2.19 ± 0.59	–
Rb	1.88 ± 0.82	–
Sr	1.21 ± 0.55	1.52 ± 0.67
Mo	–	8.14 ± 2.08

Table 4
The relative elemental contents of a cluster of epithelial cells of cervical cancer determined by SR-TXRF (unit: counts of peak area)

Element	Cancer cell before apoptosis	Cancer cell after apoptosis
Ti	158 ± 58	410 ± 71
V	709 ± 60	1456 ± 75
Fe	4177 ± 81	1837 ± 64
Cu	172 ± 38	840 ± 50
Zn	1281 ± 53	1811 ± 62
As	573 ± 39	1572 ± 53
Br	1644 ± 54	2550 ± 66
Rb	–	480 ± 55
Sr	289 ± 51	126 ± 55
Mo	855 ± 90	2675 ± 118

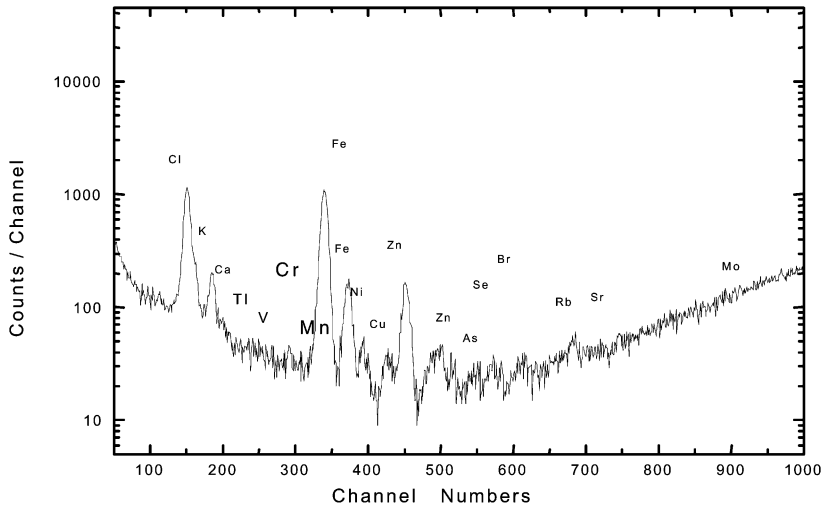

Fig. 2. TXRF spectrum of lung cancer cells before apoptosis.

homogeneous and thin, since the thickness of the residue on the polished surface is very thin (approx. a few micrometers). We used the standard reference materials of pork liver and water as the external standard. Under the same condition of XRF exciting efficiency, we have $C/C_s = N/N_s$ for the same one element, where C and C_s are elemental contents of unknown and standard samples, respectively, N and N_s are the elemental net peak counts of unknown and standard samples, respectively. In this way the elemental contents in cell specimens were preliminarily obtained and the results are shown in Table 2.

This technique was also applied to study the elemental relative contents and their changes in the cells of lung cancer and cervix cancer before and after cell apoptosis. The TXRF spectra are shown in Figs. 2 and 3 for the lung cancer cells

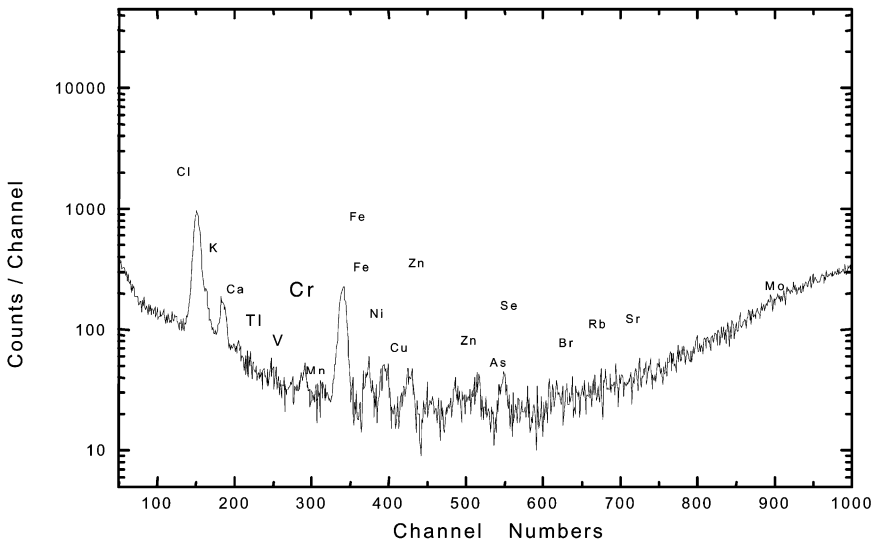

Fig. 3. TXRF spectrum of lung cancer cells after apoptosis.

before and after apoptosis, respectively. The experimental data are shown in Tables 3 and 4. In the two tables, all the measurement data were normalized to the intensity of synchrotron radiation beam current, which was monitored by the ion chamber in front of the sample. It can be seen that some elements in cancer cells vary significantly before and after cell apoptosis, these elemental changes may provide some useful information for the cure of cancer disease.

4. Conclusion

The preliminary experimental results show that the SR-TXRF experimental facility and the related method used at BSRF for analysis of trace elements in biological cells were proven to be practical. It can be seen that the SR-TXRF technique can become a powerful tool for analysis of trace elements in biological cells.

References

[1] Y. Yoneda, T. Horiuchi, Rev. Sci. Instrum. 42 (1971) 1069–1070.
[2] J. Knoth, H. Schwenke, F. Z. Anal. Chem. 301 (1980) 7–9.
[3] A. Iida, Y. Gohshi, T Matsushita, Adv. X-Ray Anal. 28 (1985) 61–68.
[4] P. Pianetta, N. Takaura, S. Brennan, W. Tompkins, S.S. Laderman, A. Fischer-Colbrie, A. Shimazaki, K. Miyazaki, M. Madden, D.C. Wherry, J.B. Kortright, Rev. Sci. Instrum. 66 (1995) 1293–1297.
[5] H.Y. Yao, J.X. Zhang, X.K. Wu, J.Q. Zhu, X.Z. Zeng, Nucl. Instrum. Methods B 75 (1993) 563–566.
[6] J.X. Zhang, P. Huang, B.J. Wu, G.F. Yu, L.Z. An, H.Y. Yao, Y.A. Xiao, J.X. Pan, Chinese J. Nuclear Med. 16 (1996) 149–151.
[7] E.S. Tang, Y.L. Yan, S.J. Xia, P.W. Wang, F.T. Yi, Y.Y. Huang, J. Liu, M.Q. Cui, J. Synchrotron Radiat. 5 (1998) 530–532.
[8] A. Iida, Y. Gohshi, in: S. Ebashi, M. Koch, E. Rubenstein (Eds.), Handbook on Synchrotron Radiation, 4, North-Holland, Amsterdam, 1991, pp. 307–348.
[9] Y.Y. Wu, Z.Y. Chao, Y.A. Xiao, J.X. Pan, E.S. Tang, Nucl. Instrum. Methods A 359 (1995) 291–294.