Spectrochimica Acta Part B 101 (2014) 98–105

Contents lists available at ScienceDirect

Spectrochimica Acta Part B

journal homepage: www.elsevier.com/locate/sab

Technical note

Application of the total reflection X-ray fluorescence method to the elemental analysis of brain tumors of different types and grades of malignancy ☆

M.W. Lankosz [a,*], M. Grzelak [a], B. Ostachowicz [a], A. Wandzilak [a], M. Szczerbowska-Boruchowska [a], P. Wrobel [a], E. Radwanska [b], D. Adamek [b]

[a] Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Krakow, Poland
[b] Department of Neuropathology, Chair of Pathomorphology, Faculty of Medicine. Jagiellonian University, Krakow, Poland

ARTICLE INFO

Article history:
Received 9 November 2013
Accepted 18 July 2014
Available online 8 August 2014

Keywords:
Total reflection X-ray fluorescence
Brain tumors
Trace elements
Grade of malignancy

ABSTRACT

The process of carcinogenesis may influence normal biochemical reactions leading to alterations in the elemental composition of the tissue. Total reflection X-ray fluorescence analysis (TXRF) was applied to the elemental analysis of different brain tumors. The following elements were present in all the neoplastic tissues analyzed: K, Ca, Fe, Cu, Zn and Rb. The results of the analysis showed that the elemental composition of a relatively small fragment of tissue represents satisfactorily the biochemical "signature" of a cancer. On the basis of the element concentrations determined, it was possible to differentiate between some types of brain tumors.

1. Introduction

The process of carcinogenesis may influence normal biochemical reactions leading to alterations in the elemental composition of the tissue. Trace elements play a significant role in neoplastic processes [1,2]. The elemental composition of neoplastic tissues, therefore, may differ from that of normal specimens and may also differentiate between various tumor types. Molecular oncology is in need of structural methods which are capable of monitoring biochemical processes and interactions within neoplastic tissues. A sort of "elemental fingerprinting" of brain tumors could provide a very useful supplementary tool during the process of diagnosing tumors in difficult or disputable cases. Synchrotron-radiation-based X-ray fluorescence elemental micro imaging hybridized with multiple discriminant analysis was used to construct a diagnostic classifier for brain tumors in the cases of various types of brain malignancy [3]. However, access to synchrotron sources is limited and the practical application of the method developed to support histopathological diagnosis is difficult and limited. The purpose of our studies was to investigate whether average concentrations of minor and trace elements in malignant tissues can be used for the differentiation and/or classification (diagnosis) of brain tumors. In the laboratory, total reflection X-ray fluorescence spectroscopy was applied to the elemental analysis of different brain tumors. The results of the analysis were evaluated using advanced statistical methods.

2. Materials and method

The samples were obtained intraoperatively from patients with glial brain tumors requiring surgical intervention. In addition, brain tissue without apparent malignant infiltration was taken. In our investigation 19 samples were used. The tumor samples were cut during the surgical procedure and frozen immediately in chemically inert vessels (Eppendorf tubes). Additional samples of tissue routinely embedded in paraffin were used for precise histopathological diagnosis, i.e. of tumor type and grade, using HE and other staining methods (if necessary also including immunohistochemistry) according to the specific requirements of each particular case. The samples were prepared and diagnosed histopathologically at the Department of Neuropathology, Jagiellonian University (UJ) Medical College in Krakow. The study was approved by the Jagiellonian University Bioethical Committee (KBET/101/B/2010). We analyzed tissues which represent various types of brain tumors whose malignancy grades were determined in accordance with the latest World Health Organization (WHO) classification [4]. This made it possible to determine the type and grade of malignancy of the tumor studied. Specification of the brain tissues used in the analysis is presented in Table 1.

☆ Selected paper from the 15th International Conference on Total Reflection X-ray Fluorescence Analysis and Related Methods, and the 49th Annual Conference on X-ray Chemical Analysis (TXRF2013), Osaka, Japan, 23–27 Sept. 2013.

* Corresponding author at: AGH-University of Science and Technology, al. Mickiewicza 30,30-059 Krakow, Poland.
E-mail address: Marek.Lankosz@fis.agh.edu.pl (M.W. Lankosz).

http://dx.doi.org/10.1016/j.sab.2014.07.019
0584-8547/

Table 1
Specification of the brain tissues used in the analysis.

Tumor type	WHO grade	Code	Number of samples
Glioblastoma multiforme	IV	GW	7
Anaplastic astrocytoma	III	GA	3
Anaplastic oligodendroglioma	III	SA	1
Oligoastrocytoma	II/III	SG	1
Atypical transitional meningioma	II	OA	1
Fibrous meningioma	I	OW	2
Metastatic carcinoma	0	RP	2
Brain tumor	0	GM	1
Cerebral abscess	K[a]	RM	1

[a] K-control sample.

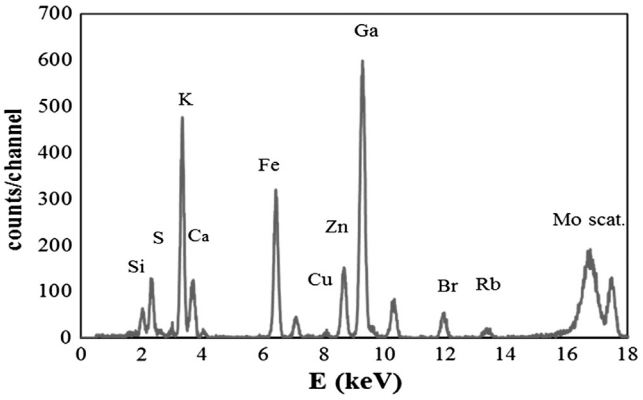

Fig. 1. Characteristic X-ray radiation excited in digested brain glioma.

Prior to analysis, the tissue samples were digested in nitric acid (Suprapur, Merck) in a pressure vessel (Parr bomb). The aim of the digestion was to remove from the tissue sample light elements such as N, C, P, and S. Frozen samples were weighed and put into a Teflon body, and then nitric acid was added. The sample masses ranged from about 29 to 440 mg. The volume of the added acid was 0.3 to 1 mL depending on the mass of the tumor. The volume of the acid was as small as possible to avoid diluting the sample. The digestion temperature was 180 °C and the digestion time was 8 h. After cooling, the bombs were opened and internal standard (Ga) was added to achieve a final concentration of 60–150 mg/kg. Samples were prepared by placing a 6 μL droplet of fluid on a microscopic slide, which was dried on a hot plate and then measured. For each tissue, three dried residues were prepared. The measurements of samples were performed using a bench-top Rigaku Nanohunter spectrometer. In TXRF measurements a Mo X-ray tube was used. In measurements regular microscope siliconized glass slides were used as reflectors. The operating voltage and current of the X-ray tube were 50 kV and 0.8 mA, respectively. The sample measurement time was 2000 s. The concentrations of measured elements were calculated using software provided by the manufacturer. A typical spectrum of X-ray characteristic lines excited in digested brain glioma is presented in Fig. 1.

3. Results and discussion

The TXRF technique revealed that elements such as K, Ca, Fe, Cu, Zn and Rb were present in all the neoplastic tissues analyzed. Moreover, in certain cases Br, Se and Sr were also detected. However, the contents of these elements in neoplastic tissues were relatively low. That is why, under the applied measurement conditions, Se and Sr were sometimes determined but not treated as an integral part of the experiment. Since Br is a volatile element, concentrations of Br were not calculated. Consequently, these three elements were excluded from further statistical analysis. The concentration of a measured element was estimated as the median of 3 measurements, whereas the uncertainty of the analysis was estimated as the range divided by the square root of 3. In order to estimate the optimal volume of droplets used for residue preparation, samples with volumes ranging from 1 μL to 10 μL were measured. The results of this analysis indicated that for residues obtained from droplets of 5 to 10 μL, the concentrations of the analyzed elements were similar. Using the spectra collected, the values of MDL were determined. The following values were obtained: K—51 ppm, Ca—40 ppm, Fe—1.8 ppm, Cu—1.2 ppm, Zn—1.3 ppm, Rb—0.5 ppm, and Sr—0.5 ppm. The precision of analysis was calculated on the basis of measurements of 10 residues prepared from the same tissue sample. The relative precision of the analysis, by element, was as follows: K—7%, Ca—43%, Fe—4%, Cu—10%, Zn—5%, and Rb—7%. The median values and uncertainties obtained for the measured elements in brain tissues are presented in Table 2.

The results presented in Table 2 indicate a wide range of variation in concentrations of elements in tissues. It is also clear that, in the case of

Table 2
Results of analysis.

Type of cancer	[K][a] [ppm]	u(K)[b] [ppm]	[Ca][a] [ppm]	u(Ca)[b] [ppm]	[Cu][a] [ppm]	u(Cu)[b] [ppm]	[Zn][a] [ppm]	u(Zn)[b] [ppm]	[Fe][a] [ppm]	u(Fe)[b] [ppm]
RM	1160	83	801	650	11.3	3.2	10.79	1.04	194	35
GM	1800	1400	19,700	8400	19.4	4,9	71	12	103.9	10.8
RP	1680	970	144	84	1.22	0.16	12.2	1.7	369	13
RP	2750	270	203	55	1.2	1.4	14.6	1.6	31.5	1.7
OW	1680	250	804	510	3.82	0.22	16.41	1.01	39.9	2.6
OW	1130	35	160	18	1.200	0.021	9.25	0.77	87.9	2.9
IOA	20,880	190	307	530	3.8	1.6	12.6	1.7	160.2	6.7
SG	1050	18	107	62	4.06	0.34	7.62	0.22	75.3	5.1
GA	860	130	910	1900	6.64	0.42	9.93	1.03	71.45	1.01
GA	2220	540	1410	41	8.9	8.4	15	14	167	14
SA	1860	81	148	14	3.01	0.42	12.67	0.97	74.2	1.5
GA	1160	75	134	49	0.91	0.65	20.39	1.04	113.7	2.9
GW	3520	580	320	160	10.3	3.4	37.3	3.4	78.98	1.02
GW	580	190	46	59	1.67	0.42	26.9	1.7	92	19
GW	1050	110	80.4	2.4	2.21	0.19	14.59	0.59	84.34	0.49
GW	2250	170	206	190	9.1	1.6	23.30	1.02	226.6	2.9
GW	2060	410	240	140	2.65	0.95	26.3	1.7	112.1	5.1
GW	1730	52	67	23	4.65	0.69	20.8	1.2	70.19	2.09
IGW	25,530	15,000	173	99	0.85	0.51	14.7	5.8	34.6	1.8

[a] Average concentration.
[b] Uncertainty.