

TRACE ELEMENT DETERMINATIONS IN LUNGS OF RATS BY NEUTRON ACTIVATION ANALYSIS

Mitiko Saiki* and Paulo H. N. Saldiva**

*Instituto de Pesquisas Energéticas e Nucleares
IPEN-CNEN/SP, Supervisão de Radioquímica
Caixa Postal 11 049, CEP 05422-970, São Paulo, Brasil

** Faculdade de Medicina da USP
CEP 01246-900, São Paulo, SP, Brasil

ABSTRACT

In this paper neutron activation analysis was applied to the trace element determinations in lungs of rats housed in regions with different levels of pollution. Lung samples were previously submitted to cryogenic homogenization, freeze drying and sterilization and they were irradiated with together the standards of elements under a thermal neutron flux of $10^{11} - 10^{13} \text{ n.cm}^{-2}.\text{s}^{-1}$ in the IEA-R1 nuclear reactor. Concentrations of Br, Ca, Cl, Cs, Fe, K, Mg, Mn, Na, Rb, Sb, Sc, Se and Zn were determined, in general, with a good precision. In order to evaluate the accuracy of the method, the NIST 1577a Bovine Liver and IUPAC Bowen's Kale reference materials were analyzed. Comparison among the results obtained for samples of rats originated from different regions was also performed.

INTRODUCTION

Characterization of trace elements in environmental samples such as particulate matter, soil, vegetation and water has provided valuable data about the general pollution level of an area, however few information concerning the real impact of air pollution on the population is available.

Consequently trace element determinations in lung samples are of great importance to study the environmental and occupational pollution effects to the health of human populations.

In order to study health effects from urban air pollution, the Laboratory of Experimental Air Pollution of Medicine School - University of São Paulo obtained several parameters about biological effects using rats exposed during six months in two heavily polluted towns: São Paulo and Cubatão[1]. Results obtained in these studies showed that rats from Cubatão seem to have been affected by pollutants with a high capacity to penetrate the lungs damaging the distal airspaces. In the rats from São Paulo, the predominant upper airway lesions, epithelial hyperplasia and mucous hypersecretions were observed indicating the presence of highly soluble pollutants with low penetration.

In this paper instrumental neutron activation analysis was used to the trace element determinations in lungs from rats housed in three regions with different levels of pollution in order to verify if there was any difference among the concentrations found depending upon the origin of the animals.

EXPERIMENTAL

Collection and Preparation of Lung Samples. Lung samples were collected from rats housed for six months in three regions of São Paulo State: Atibaia, which is a countryside town situated about 60 Km from São Paulo city and is considered as control region with clean atmosphere; Osasco, a district of São Paulo with industries situated about 20 Km from downtown and São Caetano, another industrialized town situated in the surrounding of São Paulo city. Ten to twelve lungs were taken from each region and gathered for homogenization constituting this way one sample. The cryogenic homogenization consisted of grinding the frozen samples using liquid nitrogen and drying by lyophilization for about two days. In this process there was a weight loss of about 82%. The bottles with the samples were also sterilized by using a ^{60}Co radiation source.

Instrumental Neutron Activation Analysis. About 100 mg of the samples were weighed into clean plastic envelopes and irradiated at the IEA-R1 nuclear research reactor together with the elemental synthetic standards. The synthetic standards were prepared by pipetting the elemental standard solutions onto sheets of Whatman No. 42 filter paper. Stock solutions of standards were prepared by dissolving high purity metals, oxides or salts in appropriate reagents. Diluted solutions containing one or more elements were prepared from these stock solutions.

Two separate irradiations were carried out to determine a large number of elements. Short irradiations of 5 minutes using a pneumatic system facility under a thermal neutron flux of $3.7 \cdot 10^{11} \text{ n cm}^{-2} \text{ s}^{-1}$ were performed to determine Cl, K, Mg and Na, and long irradiations of 16 hours under $10^{13} \text{ n cm}^{-2} \text{ s}^{-1}$ were done to determine Br, Ca, Cs, Fe, Rb, Sb, Sc, Se and Zn

After adequate decay time the irradiated samples and standards were fixed in stainless steel planchets and counted using a hyperpure Ge detector coupled to an EG & G Ortec 4096 channel analyzer connected to an IBM/PC microcomputer. The counting system used had a resolution (FWHM) of 1.15 keV for 122 keV gamma rays of ^{57}Co and of 2.45 keV for 1332 keV gamma rays of ^{60}Co . The gamma ray spectra were processed by using VISPECT computer program. This program evaluates peak areas(counting rates) and gamma ray energies of radioisotopes. The radioisotopes (^{82}Br , ^{47}Ca , ^{38}Cl , ^{134}Cs , ^{59}Fe , ^{42}K , ^{27}Mg , ^{56}Mn , ^{24}Na , ^{122}Sb , ^{46}Sc , ^{75}Se and ^{65}Zn) measured in this study were identified according to their half lives and gamma energies. The comparative method was used for calculating the content of the respective elements.

RESULTS AND DISCUSSION

In order to evaluate the accuracy of the method, about 200 mg of NIST 1577a Bovine Liver and IUPAC Bowen's Kale reference materials were analyzed by applying the same experimental conditions used in lung analyses. Table 1 presents the comparison between the results obtained and the certified values. Our results obtained for most of the elements agreed with the certified values and the relative standard deviations of these elements varied from 2 to 10%. Less precise results were obtained for Sb and Se present at the level of $\mu\text{g/Kg}$. The precision for Mg in Bovine Liver reference material was not so good due to the low activity of ^{27}Mg at our countings.

TABLE 1. Elemental Concentrations in IUPAC Bowen's Kale and NIST 1577a Bovine Liver Reference Materials

(Concentrations on dry weight basis)

Element	Bowen's Kale		Bovine Liver	
	This work	Ref[2]	This work	Ref[2]
Br, µg/g	24.2±2.2 (9.3)*	24.9±2.5	9.03±0.43(5)	(9)**
Ca, µg/g	40890±1889(4.6)	41060±2217		120±7
Cl, µg/g	3571±173(4.8)	3560±427	2693±131(4.0)	2800±98
Cs, µg/Kg	85±4(5.8)	76.3±5.8		
Fe, µg/g	108±2(2)	119.3±14.3	195±13(6.7)	194±19
K, %	2.42±0.15(6)	2.44±0.15	1.02±0.07(7)	0.996±0.007
Mg, µg/g	1645±49(3)	1605±176	656±89(13.6)	600±15
Mn, µg/g	14.2±0.4(3)	(14.82±1.63)	9.8±0.5(4.9)	9.9±0.8
Na, µg/g	2127±157(7.4)	2366±284	2222±124(5.6)	2430±129
Rb, µg/g	54.0±1.6(3)	53.4±5.3	12.2±0.4(3.5)	12.5±0.1
Sb, µg/Kg	64.2±7.8(12.1)	68.5±14.4	5.56±0.68(12.2)	(3)
Sc, µg/Kg	15.2±0.8(5.2)	(9.48)		
Se, µg/g	0.147±0.025(17.1)	0.134±0.020	0.60±0.07(10)	0.71±0.07
Zn, µg/g	32.9±1.5(4.6)	32.29±2.70	118±3(2.5)	123±8

* - Relative standard deviation

** - Information values

Table 2 shows the results of the arithmetical means and standard deviations calculated from at least three determinations. In this Table literature values[3] for some elements in rat lung samples are also presented.

Elements Cl, K and Na were found at the highest concentrations at the level of percentage, Br, Ca, Fe, Mg, Mn, Rb, Se, and Zn at the level of µg/g and Cs, Sb and Sc at the level of µg/Kg. These results, generally, presented good precision with relative standard deviations lower than 13.5% for most of the elements determined. For Fe, Mn, Rb and Zn, our results agreed with those presented by Sato and Kato[3].

Comparison between the results obtained for three groups of rats indicates there is no difference among the concentrations of Cl, Fe, K, Mg, Mn, Na, Rb, Sb, Se and Zn in lungs, indicating there was no accumulation of these elements from exogenous origin. These elements, breathed as gases or fine particulates, are soluble and easily eliminated from lungs by the blood stream.

On the other hand, the lowest concentrations of Br and Cs were obtained in lungs from rats housed in Osasco while the concentrations of Ca and Sc were for rats from São Caetano. Lowest concentrations of some elements found in rat groups from industrialized areas other than those found in the control group of Atibaia may be attributed to the increase of the pulmonary tissue permeability due to the pollution effects.

Being this idea valid the depuration mechanism occurs because of the lung tissue permeability and consequently there would be a reduction of biological half lives of these elements in the animal lungs.

TABLE 2. Trace Elements in Lungs from Rats Housed in Three Different Regions of São Paulo State and Literature Data.

(Concentrations on dry weight basis)

Element	ATIBAIA	OSASCO	SÃO CAETANO	Ref[3]
Br, µg/g	36±5	25±2	32±2	
Ca, µg/g	879±70	929±119	462±16	
Cl, %	1.17±0.09	1.08±0.04	1.0±0.1	
Cs, µg/Kg	174±8	129±12	199±8	Trace
Fe, µg/g	517±24	503±21	483±8	434±18
K, %	0.87±0.03	1.08±0.07	1.09±0.16	
Mg, µg/g	464±56	537±73	322±76	
Mn, µg/g	1.60±0.02	1.63±0.03	1.60±0.02	1.7±0.7
Na, %	0.71±0.05	0.72±0.03	0.66±0.01	
Rb, µg/g	47±3	38±3	40.8±0.7	36.2±6.0
Sb, µg/Kg	49±5	35±3	48±8	Trace
Sc, µg/Kg	3.8±0.2	2.4±0.2	1.6±0.2	Trace
Se, µg/g	1.34±0.03	1.33±0.04	1.28±0.04	1.87±0.20
Zn, µg/g	75±4	77±5	75±3	78±2

ACKNOWLEDGEMENT

The authors are grateful to FAPESP and CNPq for financial support.

REFERENCES

- [1] BOHN, G. M. et al. Biological effects of air pollution in São Paulo and Cubatão. *Environ Res.* 49: 208-16, 1990.
- [2] TORO, E. C.; PARR, R. M. AND CLEMENTS, S. A. Biological and environmental reference materials for trace elements and organic microcontaminants. International Atomic Energy Agency. Vienna, Report IAEA/RL.128, Rev. 1, 1990.
- [3] SATO, T. AND KATO, T. Determination of trace elements in various organs in rats by thermal neutron activation analysis. *J. Radioanal. Chem.* 53(1-2):181-90, 1979.