2nd International Symposium on Biological Reference Materials
Neuherberg, West Germany, April 24-25, 1986

**SOME UNCERTAINTIES ASSOCIATED WITH PREPARATION OF
STANDARDS IN ORGANIC MATRIX**

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ABSTRACT: Until recently no techniques which have multielement detection capability, high sensitivity and good spatial resolution in relatively thick tissue section have existed. The use of proton induced x-ray emission / PIXE / and synchrotron radiation induced x-ray emission / SRIXE / using proton and x-ray microbeams changed this situation. However there are some difficulties with the existing standards for quantitatively calibrating the trace elements concentration in biological materials. For the purpose of our experiments a special technique of standard production was applied.

INTRODUCTION

Analytical methods to characterize the composition of materials have always been important. Specifying their composition requires some knowledge of the concentrations of the elements, and sometimes of isotopes.

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of a given element on both microscopic and macroscopic scales. Because of the complexity of most problems many and various methods have to be used.

This is why much attention is paid to sample and standard preparation when measurements in the ppm / parts per million / and more recently ppb / parts per billion / ranges are attainable, and to the need to more accurate calibration techniques. No biological reference materials available so far can fulfill these requirements.

During the experiments to calibrate the lead / Cholewa et al., 1986 / and aluminium concentrations in whole-body sections at Brookhaven National Laboratory special standards were prepared.

EXPERIMENTAL

The microPIXE method was based on the 3.5 MV Van de Graaff accelerator at DNL. The experimental arrangements / Fig. 1 / for trace elemental characterization with the use of proton microbeam of the diameter of about 20 μm has been described elsewhere / Cholewa et al., 1986 /, / Bruenger et al., 1984 /.

The magnetic analysis system is used to produce a beam of monoenergetic protons which are collimated with a set of slits and then focussed to about 20 μm spot with the use of an electrostatic quadrupole triplet lens / Augustyniak et al., 1978 /. Beam currents of 20 nA can be achieved on targets.

A computer controlled stage makes it possible to move the sample with respect to the beam in 5- μm steps. A microscope makes it possible to monitor the beam position on the target during analysis. A Ortec charge particle detector, with a resolution 17.5 keV for the 5.438 MeV alpha particles from the ^{241}Am source, with a hole in its center allows measuring

the backscattered protons in order to normalize the measurements. Characteristic x-rays produced by the beams are detected with 30 mm² Si/Li/ x-ray Ortec detector with a resolution of 160 eV for the 5.898 keV Mn-K line. A complete pulse height spectrum was accumulated and stored on a hard disc and magnetic tapes in a Nuclear Data 6600 data acquisition system.

The work using the SRXRF method will be done on the X-26 beam line at the x-ray storage ring of the National Synchrotron Light Source / NSLS /. The facility will produce a collimated beam of x-ray with a diameter of 3 μ m and with an intensity sufficient to give detection limits better than 0.1 ppm for the transition elements / Gordon, 1982 /.

STANDARD PREPARATION TECHNIQUE

The main difficulty with a quantitative analysis of trace elements is to choose the right standards and make a comparison with the known concentration of the elements. There are many / eg. NBS, IAEA etc. / biological reference materials available. Their disadvantage is that they are not certified for homogeneity necessary for microanalysis purposes.

To make a quantitative analysis of lead concentration in whole-body sections 30 and 50 μ m thick, a special technique of organic standard production was applied. For this purpose a solution of lead acetate trihydrate in 10% aqueous solution of polyethylene glycol was made. Steps in the series contained 0, 0.1, 0.5, 1, 5, 10, 50, 100 and 1000 ppm of Pb by weight were prepared. In this experiment ppm was defined as micrograms of lead per ml of the solvent. About 2 ml of each concentration were put in small cellulose cups and frozen in liquid nitrogen. After that they were

placed in a large block of methylcellulose ice for mounting on the microtome / LKB model 2258 Cryomicrotome /. Frozen aliquots were cut into sections 30 and 50 μm thick and collected on Scotch 688 adhesive tape. These were put to the cryostat cabinet to dry before analysis. The procedure with standard preparation was similar to the sample preparation technique. Thus we managed to avoid systematic and methodological errors during comparison with samples.

RESULTS AND DISCUSSION

Prepared targets were analysed using microPIXE method. The results of our measurements are shown in Table 1 where the yield of number of x-rays under Pb-L peak normalized to the number of backscattered protons is given as a function of the concentration. The calculations of the area under the lines for lead were made using the PEAK code available on a ND6600 computer.

Using the criterion of Currie / Currie, 1968 / the Minimum Detectable Limit / MDL / was also determined for lead. It was about 2 ppm for a bombardment with 2×10^{13} protons. The most important conclusion is that such sensitivity was obtained on the area of about $300 \mu\text{m}^2$ for the protons with 2.5 MeV energy. To observe the homogeneity of standard production the sample was irradiated at 14 different points chosen at random. Homogeneity was about 24% between the points, which was probably due to ununiform lead distribution in the sample. Only the statistical uncertainty about 2% in the error of the concentration was taken into consideration and the standard deviation was used in the determination of the area under the Pb-L peak.

This method of standard preparation was developed at the Brookhaven National Laboratory and State University of New York at Stony Brook for a particular kind of experiments with cryosectioning biological materials. We find / Bruenger et al., 1984 / that for our purposes liquid standards deposited in the form of a drop on different kind of backings are useless. Recent works / Delbreuck-Hobarn et al., 1980 / and / Campbell et al., 1985 / questioned the use of liquid targets with internal standards for good quantitative analysis, even if the targets are fully covered by the beams, because these targets have a tendency to create a local islands where the criterion of thickness / less than 2 mg/cm^2 / for the proteins not be applied any more.

The major advantages of the propose standard production are as follows:

- Low-temperature sectioning minimizes diffusion of element in sample.
- Our technique is similar to the popular / in biology, medicine, etc. / way of sample preparation, which might help eliminate methodological errors during comparison process.
- Standard of any thickness could be prepared for most elements needed during experiment. The uniformity of production should be carefully observed.
- Use of different organic solvent makes possible modeling the matrix composition, which might be a very important factor in precise quantitative analysis.

ACKNOWLEDGMENTS AND SUPPORT

Research supported by the Processes and Techniques Branch,
Division of Chemical Sciences, Office of Basic Energy Sciences, US
Department of Energy under Contract No. DE-AC02-76CH00016, and the
National Institutes of Health as a National Biotechnology Research Resource
under Grant No. P41RR01838-02. One of us (M.C.) was a recipient of a
Fellowship from the Kosciuszko Foundation.

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FIGURE AND TABLES CAPTIONS

Fig.1 Used geometry of Si/Li/ detector, charged particles detector and target during experiments with microbeams: 1-proton microbeam, 2-charge particle detector, 3-target, 4-Si/Li/ x-ray detector.

Table 1. The number of x-rays for Pb-L peak normalized to the number of backscattered protons as a function of the lead concentration labeled in the standard.

Table 1

CONCENTRATION /ppm/	#X/#prot. $\times 10^{-6}$
5	0.35 ± 0.05
10	0.60 ± 0.05
50	3.00 ± 0.10
100	6.00 ± 0.20
1000	60.00 ± 0.50

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