PIXE MEXICO 2007

*Proceedings of the XI International Conference on PIXE and its Analytical Applications*
*Puebla, Mexico, May 25-29,2007*

# Application of Micro-PIXE and Dynamic Analysis for the Characterization of Human Hard Tissues

C.A. Pineda-Vargas[1,2*], M.E. Eisa[3], U.M.E. Chikte[4], A.L. Rodgers[5], S. Naidoo[6], J.L. Conradie[7]

[1]*Materials Research Group, iThemba LABS, P.O. Box 722, Somerset West 7129, South Africa*
[2]*Groote Schuur Hospital, Private Bag, 7935, Observatory, South Africa*
[3]*Sudan University of Science and Technology, Department of Physics P.O.Box 407, Khartoum, Sudan*
[4]*Assoc Dean, Faculty of Health Sciences, Tygerberg, 7505, South Africa*
[5]*Head Chemistry Department, University of Cape Town, Rondebosch, South Africa*
[6]*Faculty of Dentistry, P/Bag X1, Tygerberg, 7505, South Africa*
[7]*Accelerator Group, iThemba LABS, P.O. Box 722, Somerset West, 7129, South Africa*

**Abstract.** In recent years applications of PIXE in the bio-medical field have opened important areas of research with a significant sensitivity to compete with techniques such as XRF, EDXRF, EMP and/or SEM. The micro-PIXE technique is used to investigate the morphology as well as the elemental composition of human tissues on a microscopic scale. In addition, complementary ion beam techniques are used to provide information on the major and minor components. This paper will deal with an overview of the bio-medical projects at iThemba LABS/GSH particularly in relation to spatial distribution of trace metals in hard human tissues such as renal stone concretions, teeth and hair, undertaken at the Nuclear Microprobe (NMP) facility. Relevant information about ion beam techniques used for material characterization will be discussed. Dynamic Analysis (DA) was used to extract information on spatial distribution of trace elements (TE) within selected micro-regions. This will be illustrated with several examples using proton energies of 1.5 and 3.0 MeV and applied to: 1) Teeth erosion mechanisms; 2) Human renal stone concretions nucleation region analysis; 3) Mapping of human hair cross sections from two different population groups.

* *Corresponding Author*
**Keywords:** Proton microprobe, PIXE, Calcium Oxalate, Stone concretions, Teeth erosion, human hair.
**PACS** number(s): 87.64.-t; 87.90.+y

## INTRODUCTION

Analysis for activation using protons of 1-3 MeV has been given a substantial step forward with the improvements of the micro-analysis mode and development of sophisticated software for on/off line deconvolution of x-ray spectra [1,2]. Some of the field in which nuclear microprobe (NMP) has been most powerful is in bio-medical studies for which the low levels of detection limits combined with the availability of multi-techniques and multivariate capabilities has proven to be the instrument of choice in many applications[3,4]. Furthermore, needs to explore smaller regions of bio-medical materials and tissues to the micro and nano scales has prompted the X-ray microscopist to consider the technique of elemental mapping by micro-PIXE with proton beam probes of the order of 1 μm.

In recent years applications of PIXE in the bio-medical field have opened important areas of research with a significant sensitivity to compete with techniques such as XRF, EDXRF, EMP and/or SEM. The micro-PIXE technique is used to investigate the morphology as well as the elemental composition of human tissues on a microscopic scale. In addition, complementary ion beam techniques are used to provide information on the major and minor components [4,5]. This paper will describe an

overview of bio-medical projects particularly in relation to spatial distribution of trace metals as evaluated by the technique of Dynamic Analysis (DA) [6] in hard human tissues such as renal stone concretions, teeth and hair, undertaken at the NMP facility, iThemba LABS.

## EXPERIMENTAL CONSIDERATIONS

### Beam quality and reliability for high-resolution NMP analysis at the Van de Graaff Accelerator

The NMP facility at iThemba LABS [7,8] is equipped with detection instrumentation to be able to undertake simultaneous analysis with PIXE, proton-BS and PIGE. In addition a detector located at $0^0$ to the beam line can be used to record the STIM spectrum on and off-axis of thin layers of bio-medical tissues to determined areal density variation within tissues. Recent re-engineering of the 6 MeV single ended Van de Graff accelerator at iThemba LABS has facilitated the routine optimization of target beam currents of the order of 10-100 pA and the long term reliability of the NMP for analysis in a wide variety of materials, particularly Ca-rich bio-medical tissues where fine tuning of critical experimental parameters is required.

Recalculation of beam optics from the ion source in the terminal through the accelerator and beam line up to the NMP and installation of two additional quadrupole magnets and a set of object slits, at the exit of the accelerator have contributed to marked improvement in energy resolution from 1% to 0.12% [9]. Improvements in the pumping speed of the electrostatic lens system volume have resulted in the variation of beam intensity to be less than 2%. These improvements resulted in a remarkable increase in the reliability and stability of the beam for proton microprobe work.

### Sample preparation

An important step in the analysis by NMP of soft bio-medical specimens in general is their adequate preparation for analysis under high vacuum conditions. Typically this will involve strict protocols for samples preparation at low temperature. In addition, a "true" representation of elemental concentrations is critical and this depend to a large extent on the optimal protocol used [3,10]. On the other hand biological hard tissue does not require in general the use of cryogenics since the mobility of ions in such hard materials is minimal. Therefore this paper will concentrate on the advantages of the NMP for the analysis of human hard tissues prepared at room temperature. The steps for preparation of each tissue described briefly in this paper will be explained on the corresponding section.

### Optimization of beam energy, absorber, charge collection and detector solid angle

As in any other instrumental analytical technique the optimization of experimental parameters in micro-PIXE to obtain the best minimum limits of detection (MDL) play a major role in the determination of trace elements present in hard tissue. Particularly since most of such tissues are rich in calcium and phosphorus. For trace elements such as Mg, Na, Al, Si and Al present in a calcium-rich matrix an absorber must be chosen to minimize the high background due to secondary electrons as well as to reduce the high intensity X-ray signal from Ca and P. However this is not an easy task when considering the proton energy for X-ray excitation as well as the detector exposure to scattered protons through the absorber. On the other hand this high intensity background can also be generated due to a high target current. Therefore a compromise has to be reach between these three parameters in order to be able to maximise the MDLs. The problem is rather less complicated when higher proton energies ($2.5 < Ep < 3.0$ MeV) are used for determination of heavier elements such as Ca, Ti, Mn, Fe, Ni, Cu, Zn and Br, since the insertion of a thicker absorber will control both the background and the high matrix signal. By experience we have found that for low energy protons ($1.0<Ep<1.5$ MeV) a Be absorber 25 μm thick works well; and for high energy protons bombardment ($2.5<Ep<3.0$ MeV) an Al absorber 102 μm thick is most suitable. As far as the beam current used for analysis of hard tissue it all depends on the type of material. As a general rule we irradiate the samples in such a way to prevent evaporation of elements to maintain the true composition of materials stable while under bombardment. Typically a current of between 50-300 pA is required depending on the type of hard tissue and the size of the micro-region irradiated. This level of intensity will ensure that a true non-destructive analysis is performed in most samples. Total charge integrated will depend on the levels of trace elements of interest. PIXE two critical parameters – solid angle subtended by the Si(Li) detector and thickness of absorber positioned between the specimen and the detector – are routinely calibrated using standard reference materials of pure metals and minerals supplied by Astimex®.

## APPLICATIONS ON HUMAN HARD TISSUES

### Elemental mapping of teeth enamel-dentine interface exposed to acidic conditions:Average Elemental

Investigations on teeth erosion with 1.5 MeV protons [5] revealed certain areas of depletion of major

components Ca and P in and around the interface enamel-dentine. Since this dissolution may include the depletion of TE associated with morphological changes [11], an in-vitro investigation into the role of TE in erosive processes in enamel-dentine boundary was undertaken with particular emphasis in the detection and quantification of mineral loss in early artificial erosion lesions. The process of demineralization in teeth erosion due to exposure to acidic media was investigated by PIXE with 3.0 MeV protons, in a group of test and control healthy human molar teeth. Samples were cut in cubes of ~2 mm side prior to exposure for 20 h in a 3.6 pH solution. A control group was also exposed to a neutral solution (threshold pH 5.6) with pH value in the range 5.6 – 6.0, for the same period of time. All samples were thick for the 3.0 MeV protons with average effective range of analysis ~50 mg/cm$^2$ for most of elements. The assumed composition of the teeth matrix was apatite. Atomic ratios of major components O, Ca, P, and F were determined by simulation of p-BS spectra using RUMP [12]. Typical values of major components atomic fractions for a set of analysis in either the dentine and/or the enamels in the control group were similar: $O_5P_1Ca_3F_1$, as compared to the test group in which these fractions values were: $O_6P_{0.5}Ca_3F_{0.5}$ for both enamel and dentine.

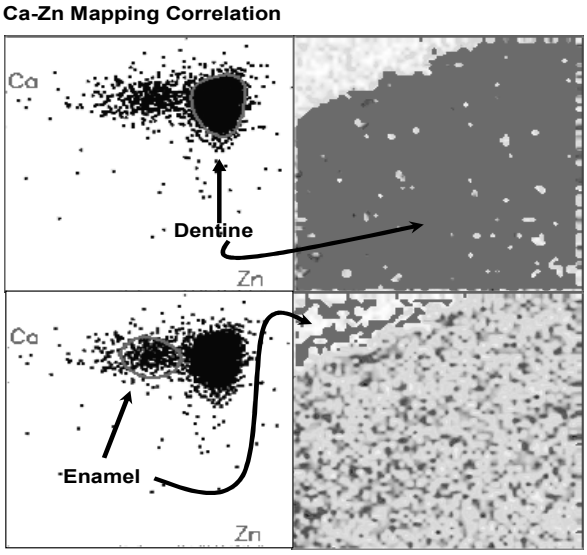


**FIGURE 1.** Elemental Mapping distribution of a dentine control sample over an irradiated enamel-dentine interface. 2-dimensional correlations of the 64 x 64 pixels (scan size ~1000 μm$^2$) square data matrix for maps of Ca and Zn (shown on the left of the figure) gave two kinds of distributions, each associated with either the dentine or enamel area indicated by different gray scales. The trend of the interface boundary is also visible. The weakest correlation corresponds to that on the enamel area [13].

Elements analysed by micro-PIXE were Mn, Fe, Ni, Cu, Zn, Sr and Sn for the controls and tests samples on dentine and enamel. Since two halves of same teeth specimen was used as test and/or control it could be assumed that the composition of the controls can be assigned to TE present in both the enamel and/or the dentine. On the other hand observing the concentration level for Sr in the controls (~140 μgg$^{-1}$) it was observed that after treatment their average concentration was reduced to ~80 μgg$^{-1}$. This is a significant reduction, which occurred in all tests samples independent of being enamel or dentine. A similar situation appears to be occurring for Zn, although to a lesser extent, since there were two test enamels for which the Zn levels rather increased substantially. Being Zn and Sr two biological important TE in relation to the structure of the teeth it is logical to think that the depletion of such elements after the exposure to the acidic solution treatment is related to an erosive process. Since an opposite situation did occur for Fe and Cu it is possible that a re-mineralisation may also occur [13].

Elemental quantitative maps obtained by DA [6] for Sr and Zn showed that their distribution levels over the area comprising the interface enamel-dentine were lower for the enamel. This applies particularly for specimens after erosion treatment. The depletion of Sr may act as a protectant to demineralization, contrary to the increase in certain elements such Fe and Ni. Although it's not clear why there appear to be a re-mineralisation process for these two elements it is envisaged that their role in erosion is not well understood. Figure 1 shows the mapping over an irradiated interface of a dentine control sample. The mapping correlation between Ca and Zn on the left of the figure clearly shows two kinds of correlation distributions, each associated with either the dentine or enamel area. The trend of the interface boundary is also visible. The weakest correlation corresponds to that of enamel.

## Calcium-rich kidney concretions - comparison between two population groups

Studies on the determination of TE in human renal stones by nuclear microprobe [14,15] have shown the strength of NMP application to focus on determining levels of variability in elemental concentration of Ca and TE throughout selected micro-regions of renal stones. A more recent study has concentrate on the micro analysis of two set of renal stones from two distinct population groups from Sudan and South Africa [16]. The emphasis was on a statistical comparison of the two groups in terms of their elemental profile content by country and to establish if a general trend in terms of elemental variability could be establish based on data obtained from micro-PIXE and p-BS.

Kidney concretions varied from small (~1-3 mm) to big (~1-3 cm). Their matrix composition (as determined by proton-BS, was in general calcium oxalate monohydrate (COM) and/or dehydrates (COD). The Sudanese group had matrices, which exhibit a combination of both COD and COM, but in addition some of the stones areas were rich in other type of phases such as uricite. Clinically removed concretions were embedded in epoxy at room temperature. Cross sections through the core were cut with a diamond saw.

Major components C, O, N and Ca were determined by p-BS and phase identification with powder XRD. Of interest was the fact that some of the stone phase identification did not correspond to the p-BS ratio evaluation. This could be explained by the different in size probed by the proton probe in PIXE and the X-ray signal in XRD. TE were determined by PIXE. The analyses were directed primarily to the core of the concretions. Therefore the concentrations by PIXE are related to the particular phase found within the core of each renal stone. In general the spatial distribution of Ca in most stones was non-uniform. Micro-PIXE results were aimed at the spatial distribution of Fe, Ni, Cu, Zn and Sr for both groups.

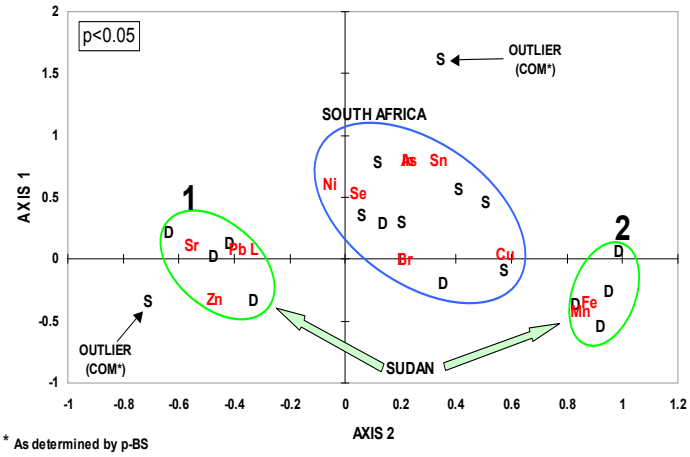


**FIGURE 2.** Correspondence Analysis of the first two axes for the two groups of renal stones from Sudan (D) and South Africa (S). Stones from South Africa are clearly clustered into one group. One outlier (S) has elemental profile that resembles mostly the group 1 from Sudan [16].

Mean values of the concentration levels for each TE in each renal stone country group were plotted to determine the level of correlation between the mean trace elemental profiles of each group. This relationship showed a type of linear correlation between mean profiles. In particular it was observed two kind of distributions governed in one hand by Zn, Fe, Sr and Ni, Cu, Br on the other hand. Therefore the data matrix for elemental concentrations by PIXE of all stones as determined by was submitted to correspondence analysis (CA) [17,18]. Figure 2 shows the plot of the CA for the first two axes, which contains approximately 80% of all the statistical information. The South African stones were tight grouped near the origin of the CA plot and are mostly defined by Cu, Br and Sn, with a high correlation between Cu and Br. Two stones were found to be outliers with one sample correlated with the Sudanese renal stones. The Sudanese stones were separated into two subgroups, characterized by high levels of Sr (sub-group 1) and Fe (sub-group 2). Two stones from Sudan in particular were found to be correlated with the South African group. Although the 2 subgroups of stones from Sudan originated from two different regions within Sudan, the grouping as shown in Figure 2 does not appear to be associated to the geographical location. On the contrary, Sudanese stones were grouped according to sub-groups 1 and 2. These two sub-groups contain Sudanese stones from both locations.

## Analysis of human hair cross sections

Elemental analysis of human hair has been established as an important screening technique to assess body-nutrient levels associated with poor health conditions and/or toxicity due to environmental pollutants particular for long periods of time. In spite of the great number of research analysis reported on this material by a wide variety of instrumental analytical techniques relatively little is being done with IBA techniques, particularly with micro-PIXE [19]. With the aim to compare metal content and spatial distribution of hair in two distinct human population groups from Sudan and South Africa, microanalysis was performed. Hair sample material from healthy male South African (average age 36 years) and Sudanese persons (average age 35 years) living in urban areas was collected for analysis. Hair samples were embedded in resin and thin cross sections (~ 5 μm) were cut in a microtome.

Elemental matrix composition of hair cross sections for major components founded by RUMP [12] showed a similar trend in both population groups. With average composition $C_7O_{0.6}N_2S_{0.1}$ and $C_8O_{0.7}N_2S_{0.1}$ for the South African and Sudanese groups respectively. Concentration of S, K, Ca, Fe, Ni, Cu, Zn and Sr, were obtained by irradiation with 3.0 MeV protons. A South African sample in particular had very low concentrations of S, Ca and Zn as compared with the other South African and Sudanese hair samples. The Sudanese group on the other hand appear to be more homogeneous as far as trace and major components is concerned. Elemental maps reconstructed by DA from irradiations with 1.5 MeV protons showed differences in the spatial distribution of certain elements such as S, K, Ca and Zn in the cuticle, cortex and medulla areas of the hair cross sections (see Figure 3)[20]. Elemental data for PIXE

and p-BS evaluated with correspondence analysis [17] showed that the Sudanese group appeared to form a tighter cluster while the South African group was scattered forming an elongated group. This elongation was determined primarily by a South African sample which had a low content of S, Ca and Zn.

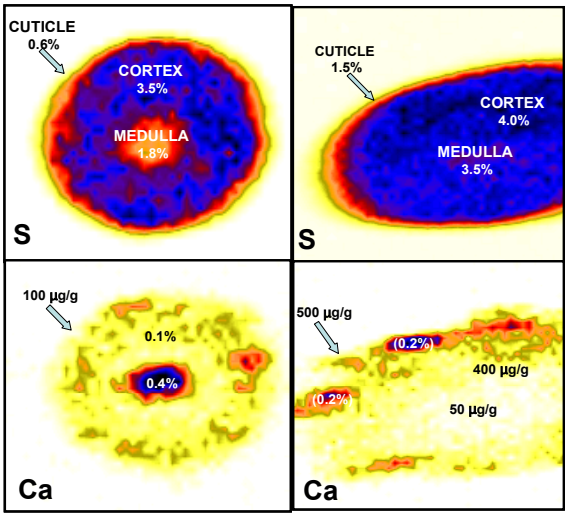


**FIGURE 3.** Maps of sulphur and calcium for a typical Sudanese (left) and South African (right) hair cross-sections. The range values in all three areas (cuticle, cortex and medulla) are indicated in the figure. Note the inverse correlation of these two elements in the medulla region. Sulphur appears to be distributed similarly in both samples [20].

## DISCUSSION AND FINAL REMARKS

In the micro analysis of small areas of hard human tissues such as teeth and hair as well as tissue resulting from pathological conditions such kidney concretions it is of extreme importance the optimization of experimental parameters which will determine the accuracy and minimisation of the minimum detection limits. Recently there has been a renewed interest in the application of nuclear microprobe in the bio-medical field, particularly for specimens from hard human tissues. This has been demonstrated above with the discussion on three examples of research currently carried out at our institute. The current output of research is encouraging and further investigation in a wide range of materials and population is envisaged for the future. Dynamic Analysis has proved to be a strong combination for the determination of elemental correlations otherwise hidden in a normal PIXE analysis. The last point refers to the possibility of identification of different phases by the correlation of 2-dimensional data from reconstructed maps as is the case in the example of Ti implants. Further on, the possibility of reaching nano size levels for the proton probe open new avenues for the application of NMP and DA in the new emerging field of nanotechnology applied to medicine.

## ACKNOWLEDGMENTS

This work was partially supported in South Africa by National Research Foundation grant number 63509. The staff of the MRG at iThemba LABS is also thank for their support.

## REFERENCES

1. G. Ryan, D.R. Cousens, *Geo-PIXE II Quantitative PIXE trace element analysis and imaging*, CSIRO Exploration and Mining, North Ryde, NSW 2113, Australia, 2002
2. C.G. Ryan, E. Van Achterbergh, C.J. Yeats, T.T. Win, G. Cripps, *Nucl. Instrum. and Meth.* **B 189**, (2000) 400-407.
3. Y. Llababor, Ph. Moretto, *Nuclear microprobes in the life sciences,* Singapore, World Scientific, 1998.
4. C.A. Pineda-Vargas, V.M. Prozesky and W.J. Przybylowicz. *Nucl. Instr. and Meth.* **B 181** (2001) 493-498.
5. V.M. Prozesky, C.A. Pineda, J. Mesjasz-Przybylowicz, W.J. Przybylowicz, C.L. Churms, K.A. Springhorn, P. Moretto, C. Michelet, U. Chikte, P. Wenzl. *Nucl. Instr. and Meth.* **B161-163** (2000) 852-859.
6. C.G. Ryan, D.N. Jamieson, C.L. Churms, J.V. Pilcher, *Nucl. Instr. and Meth.* **B 104** (1995) 157-165.
7. V.M. Prozesky, W.J. Przybylowicz, E. Van Achterbergh, C.L. Churms, C.A. Pineda, K. Springhorn, J.V. Pilcher, C.G. Ryan, J. Kritzinger, J.H. Schmitt and T. Swart, *Nucl. Instr. and Meth.* **B104** (1995) 36-42.
8. W.J. Przybylowicz, J. Mesjasz-Przybylowicz, C.A. Pineda, C.L. Churms, C.G. Ryan, V.M. Prozesky, R. Frei, J.P. Slabbert, J. Padayachee and W.U. Reimold, *X-ray Spectrometry*, **30** (2001) 156-163.
9. J.L. Conradie, M.E. Eisa, P. Celliers, H. Delsink, D. Fourie, G. De Villiers, P.M. Maine, K. A. Springhorn and C.A. Pineda-Vargas, *Nucl. Instr. and Meth.*, **B 231** (2005) 101-105.
10. M.A. Hayat, *Princilples and techniques of electron microscopy: Biological aplplications*, 4th Ed., U.K., Cambridge University Press, 2000
11. A. Millward, L. Shaw, A.J. Smith, *Journal of Oral Rehabilitation*, **22** (1995) 37-42.
12. L.R. Doolittle, *Nucl. Instr. and Meth.* **B 15** (1986) 227-231.
13. C.A. Pineda-Vargas, A.L. Rodgers, M.E. Eisa, Rad. Phy.&Chem, **71** (2004) 947-950.
14. C.A. Pineda, M. Peisach,. *Nucl. Instr. And Meth.* **B 85** (1994) 896-900.
15. C.A. Pineda, A.L. Rodgers, V.M. Prozesky, W.J. Prybylowicz,. *Cell. Mol. Biol.*, **42/1** (1996) 119-126.
16. C.A. Pineda-Vargas, M.E. Eisa, U.M.E. Chikte, J.L. Conradie, *Rad. Phy.&Chem.*, **71** (2004) 937-942.
17. M.J. Greenacre, *Theory and applications of correspondence analysis,* Academic Press, London, 1984.
18. StaSoft, Inc. STATISTICA Data analysis software system, Version 6.1 www.statsoft.com, 2003.
19. V. Valcović, M. Jakšić, F. Watt, G.M. Grime, J. Wells and J.W. Hopewell, *Nucl. Instrum. and Meth.*, **B 75** (1996) 173-176.
20. C.A. Pineda-Vargas *et al.*, (submitted for publication).