Chapter 4

Materials and Methodology

Introduction

This study is focused on the methods to trace the elements such as lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), and magnesium (Mg) as well as calcium (Ca) in human teeth. Two methods based on the theory of X-ray fluorescence (XRF) and inductive coupled of plasma-atomic emission spectroscopy (ICP-AES) had been employed here. However, XRF method was used only in detecting the Pb due to the limitation of suitable excitation sources for Cd, Zn, Cu, Mg and Ca.

4.1 XRF: Instrumentation

Two main components are used in the XRF experiment, which are surface barrier detector supplied by Amptek associated with Quantum multi-channel analyzer (MCA) and a radiation source (Technetium-99m, as an excitation source with the gamma ray energy of 140.5 keV).

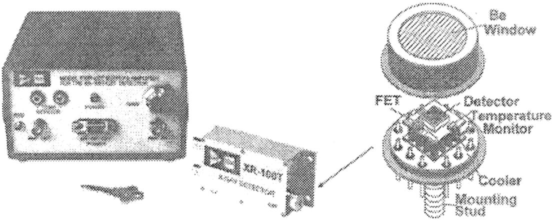


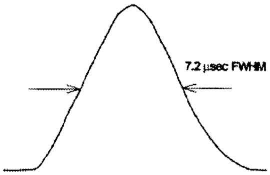
Figure 4.1: The XR-100T-CZT Detector

The XR-100T-CZT detector employs highly advanced technology, which is very sensitive to X-ray and gamma ray. This XR-100T-CZT provided "off the shelf" performance previously available only from expensive cryogenically cooled systems. This detector is supplied with Preamplifier and Cooler Systems by using a 3 x 3 x 2 mm^3 Cadmium Zinc Telluride (CZT) and mounted on a thermoelectric cooler. The input FET and feedback components to the Amptek A250 charge sensitive preamp are also mounted on the cooler.

The temperature of the internal components are kept at approximately -30^0C ($\sim 250^0K$), and can be monitored by a temperature sensitive integrated circuit. (Sensitivity: 1μA corresponding to 1^0K). The detector's surface has a light tight and vacuum tight 10 mils (250μm) Beryllium window.

All the critical connections between detector and preamplifier have been made internally to the detector to ensure quick, first time operation by the user. This detector is also provided with BNC connectors and power cable.

The Preamplifier (PX2T) was developed to provide the DC voltages needed to operate the detector, and the signal processing through the Shaping Amplifier and Rise Time Discrimination (RTD) circuits. The signal output from the Preamplifier can be connected directly to a Multi-channel Analyzer (MCA). Figure 4.2 shows the amplifier output signal. Figure 4.3 shows the circuit design and connections between the AXRT-CZT hybrid sensor, as well as the external connections to the PX2T.



Vert: 1 Volt/div **Horiz: 2 µsec/div**

Figure 4.2: PX2T-CZT Amplifier Output

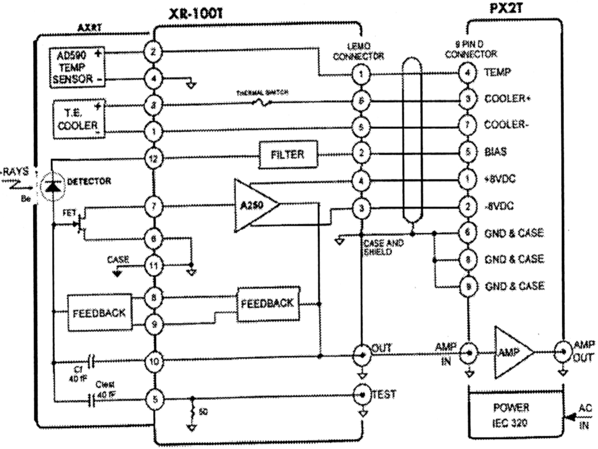


Figure 4.3: XR-100T-CZT Connection Diagram

4.2.1: The Detector

The detector used is based on the transformation of the particle energy into electric pulses occurring at the junction region of a semiconductor material, such as silicon and germanium.

The X-rays or gamma rays interact with cadmium zinc telluride (CZT) atoms to create an average of one electron / hole pair for every 5.0 eV of energy lost in the CZT. This process depends on incoming radiation, so, the energy lost is dominated by either the photoelectric effect or Compton scattering. The probability or efficiency of the detector to "stop" the incoming radiation and create electron / hole pairs increases with the thickness of CZT.

In order to facilitate the electron / hole collection process in the CZT detector, a 400-Volt potential is applied. This voltage is too high for operation at room temperature, as it will cause excessive leakage, and eventually a breakdown. However, by cooling the detector, this leakage current will be reduced considerably, thus permitting the high bias voltage.

The X-rays or gamma rays create the electron / hole pairs and interacts with CZT near the back contact of the detector, will result in fluctuations of charge collection times. These fluctuations are observed as rise time variations of the voltage step at the output of the charge sensitive preamplifier. As a result, the acquired spectra suffer from increase background counts and degraded energy solution. To reduce these effects, a Rise Time Discrimination (RTD) circuit has been developed. When the RTD is active, the shaped pulses are internally gated and only pulses corresponding to "full charge collection" events are allowed to be sent to the Multi-channel analyzer (MCA).

The thermoelectric cooler plays an important role by cooling both the CZT detector and the input FET transistor to the A250 charge sensitive Preamplifier. Cooling the FET reduces its leakage current and increases the transconductance, which in turn reduces the electronic noise of the system.

The electronic noise has to be further minimized by placing the feedback capacitor and part of the current feedback network to the A250 preamp on the same substrate as the detector and FET. This can reduce parasitic capacitance at the input.

A temperature monitoring integrated circuit is placed on the cooled substrate to provide a direct reading of the temperature of the internal components, which will vary with room temperature. Once the internal temperature gets below minus 10^0 C, the performance of the XR-100T-CZT will not change with a temperature variation of a few degrees. Hence, accurate temperature control is necessary when using the XR-100T-CZT inside the laboratory. Figure 4.4 shows the detection efficiency for the CZT detector over the energy from 0 to 1000 keV. Obviously the efficiency decreases as the detection energy increases.

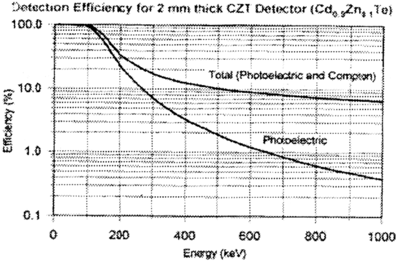


Figure 4.4: Detection Efficiency for 2 mm thick CZT Detector ($Cd_{0.9}Zn_{0.1}Te$)

4.2.2: Multi-channel Analyzer (MCA)

A basic components and function:

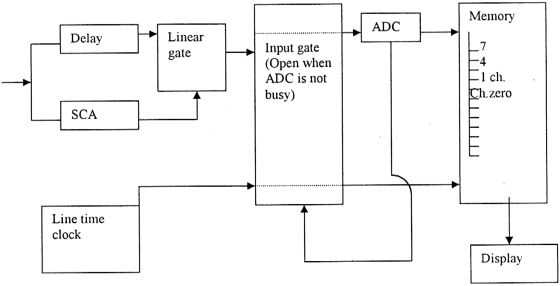


Figure 4.5: A Simple Schematic Functional Block Diagram of A Typical MCA

The operation of the MCA is based on the principal of converting an analog signal (the pulse amplitude) to an equivalent digital number by analog-to-digital converter (ADC). The output of the ADC is stored in a computer-type memory, which has as many addressable locations as the maximum number of channels of the recorded spectrum can be subdivided.

The memory can be arranged as a vertical stack of addressable locations, which ranges from the first address (or channel number 1) at the bottom through the maximum location number at the top. Once a pulse has been processed by the ADC, the analyzer control circuits seek out the memory location corresponding to the digitized amplitude stored in the address register, and the content of that location is incremented by one count. The net effect of this operation is the pulse, which have to be analyzed while passing through the ADC and is sorted into memory location that corresponds most closely to its amplitude.

This function is neglecting dead time, each input pulse is taking into account in memory location as one count. Therefore, the total accumulated number of counts over all memory is simply the total number of pulses presented to the analyzer during the measurement period. A plot of the content of each channel versus the channel number will be the same representation of the differential pulse height distribution of the input pulses.

The similarity of the PCA components to those of the standard personal computer (PC) allows the standard PC to be converted into a MCA by the plug in cards. ROI (Regions of Interested) allows the summation of selected portions of the spectrum. It also provides the indicate cursors for indicating the position on the displayed spectrum. The upper and lower bounds of the channel numbers in between give the summation counts of the spectrum. The operation is of obvious practical use for peak area determination in radiation spectroscopy. Other operations, such as subtraction of a spectrum stored in one half of the memory from the contents of the second half, are also available on some preprogrammed analyzer.

For this study, Quantum MCA Leap had been used together with the detector to set-up parts of the XRF system.

4.2.3 A Measurement on Standard Radiation Sources of Ba-133, Am-241 and Cs-137

Prior to carrying out the XRF experiment on Pb (lead), a number of measurements was done on some standard radiation sources, namely Ba-133, Cs-137 and Am-241 in order to test and calibrate the detector. Figure 4.6, 4.8 and as well as 4.10 shows the measurement spectrum of Ba-133, Cs-137 and Am-247 respectively. These figures have been used to compare with the spectrums from Amptek manual as shown in figure 4.7, 4.9 and 4.11 for Ba-133, Cs-137 and Am-241 respectively. Figure 4.6 shows the spectrum of Ba-133 with the measurement time of 6183 seconds. The measurement peaks of Ba-133 is clearly presented as shown in Amptek manual (figure 4.7), with both of them presented in log scale on the y-axis.

Figure 4.8 shows the spectrum of Cs-137. This measurement spectrum did not clearly show the peak of 662 keV if compared with figure 4.9.

Figure 4.9 shows the spectrum of Am-241. The peak of energy 59.5 keV was noted as shown in figure 4.11.

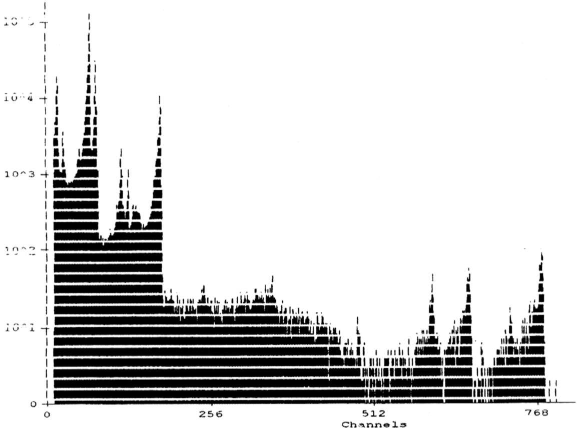


Figure 4.6: A Measurement Spectrum of Ba-133, with the Amplified Gain of PX2T at 0.5 with the counting duration time of 6183 seconds. (Y-scale: Log)

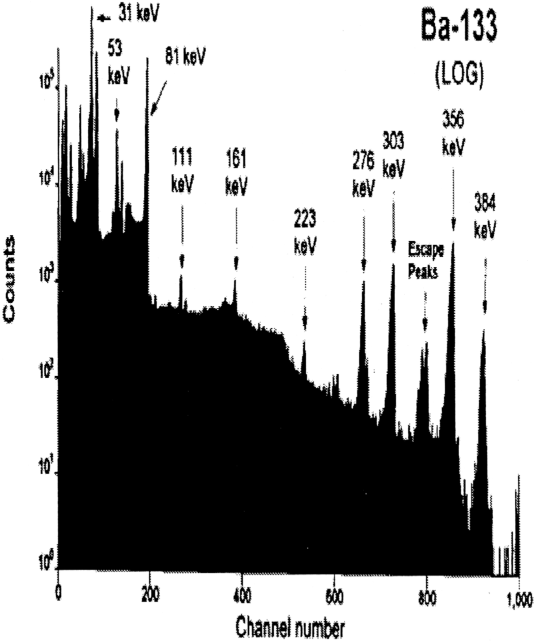


Figure 4.7: Standard Spectrum of Ba-133 (from Amptek manual)

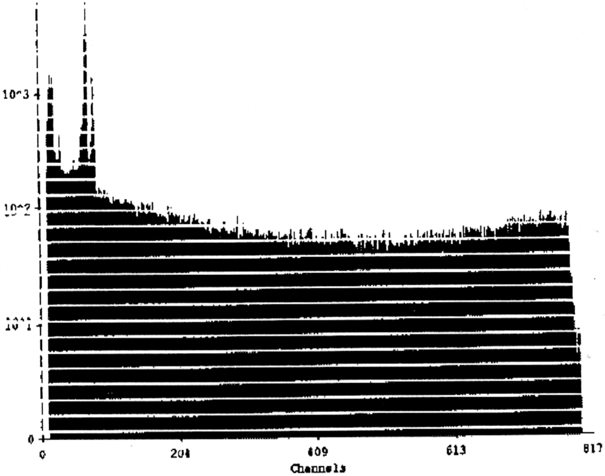


Figure 4.8: A Measurement Spectrum of Cs-137, with the Amplified Gain of PX2T at 0.5 with the counting duration time of 8572 seconds. (Y-scale: Log)

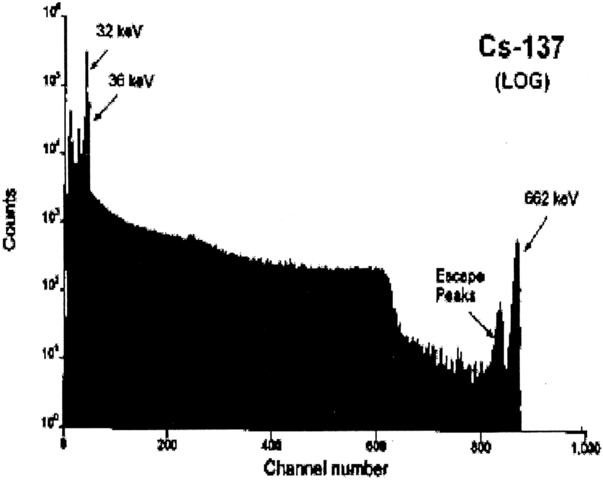


Figure 4.9: Standard Spectrum of Cs-137 (from Amptek manual)

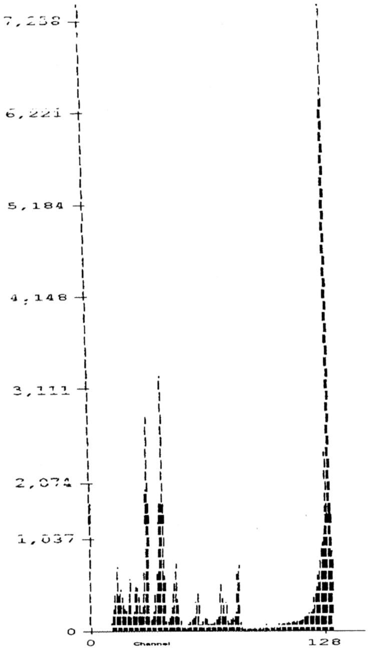


Figure 4.10: A Measurement Spectrum of Am-241, with the Amplified Gain of PX2T at 0.5 with the counting duration time of 6500 seconds.

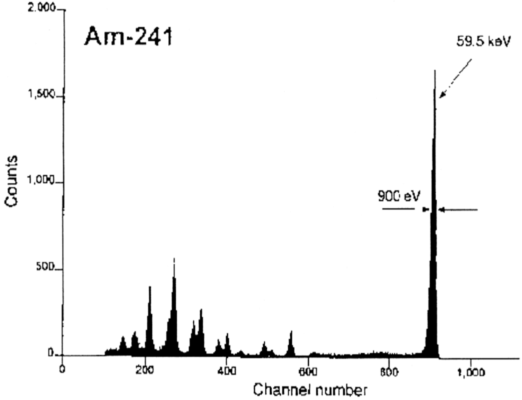
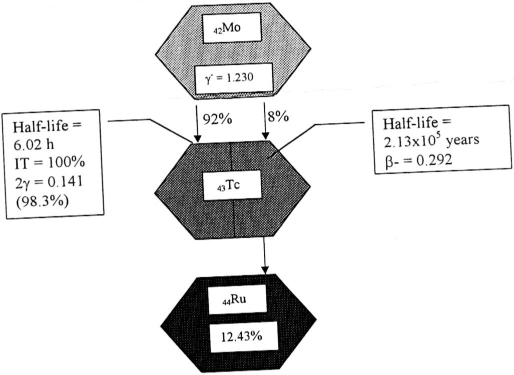


Figure 4.11: Standard Spectrum of Am-241 (from Amptek manual)

4.3 Technetium-99m (99m-Tc)

Tc-99m is chosen as an exciter source for this study because of the availability from the local hospital.

The 99m-Tc generator was set-up and is based on the adsorption of carrier-free-99-Mo on an alumina (Al_2O_3) column. The 99-Mo activity in the form of molybdate ion, MoO_4 is bound to an alumina column. The 99m-Tc activity, being chemically different, is not bound by the alumina and is eluted from the column with 5-25 ml of normal saline. Typically 75-85 percent of the available 99m-Tc activity is extracted in a single elution, with the maximum activity about 24 hours later. 99m-Mo generators are useful for about a week due to the 66-hour half-life.



99-Mo is produced with two different methods. The first (and cleanest) is the neutron activation of stable 98-Mo through the (n, gamma) reaction. However, this method results in a lower specific activity.

The half-life is 6.02 hours for 99m-Tc is a 1/2-energy state. The decay mode(s) are internal conversion and beta decay. The beta transition is 3.70E-05 in yield to stable 99-Ruthenium as shown in figure 4.12(a). The remaining transitions are internal transition to the 7/2+ state (99.24%, about 2 keV) and the 9/2+ state of 99-Tc (0.76^, 322keV), which is a beta emitter with a half-life of 200,000 years. It is noted, that the internal conversion to the 7/2+ state accounts for the vast majority of the transitions, and this internal transition involves M and N shell electrons, making this metastable nucleus particularly susceptible half-life changes due to chemical environment. The change is of the order of 0.3% when the isotope is in the Tc2S7 compared with the KTcO4 molecule. From the 7/2+ state the nucleus decays promptly to 9/2+ (99-Tc), with 89.1% of the transitions resulting in the familiar 140.5 keV. The isomeric level decay of Tc-99m was shown in figure 4.12(b). The spectrum of Tc-99m is shown in figure 4.13. Thus, it is seen that for every 99-Mo decay, approximately 85% result in (delayed) 140.5 keV gamma ray transitions.

Tc-99 is a pure beta emitter. This nuclide is useful for thickness gauge of film and leather. Tc-99m is used extensively in medical nuclear imaging.

Figure 4.12 (a): Technetium-99 Beta Decay:

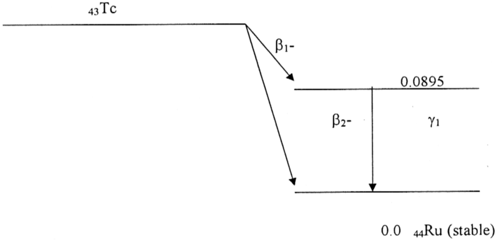
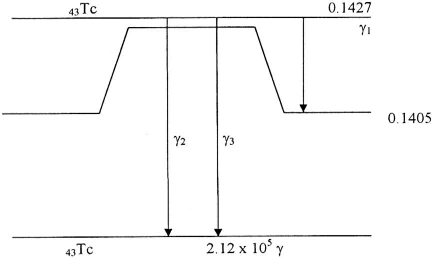


Figure 4.12 (b): Technetium-99m Isomeric Level Decay:



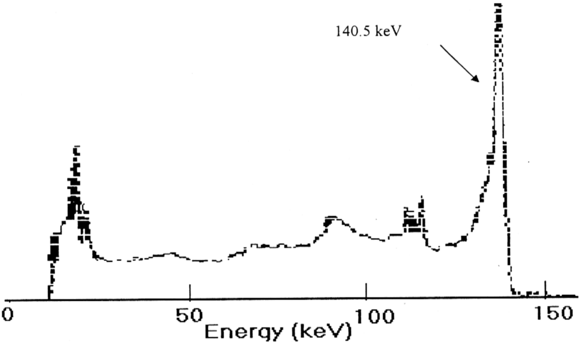


Figure 4.13: Spectrum of Tc-99m (with activity of 3 mCi)—Counting time of 1340 seconds

4.4 Materials Preparation for XRF Experiment

The XRF experiment had been carried out with the main purpose to determine the minimum concentration of lead, which could be detected by using Tc-99m and the XR-100T-CZT set-up. Therefore, all possible means of contamination have to be eliminated during the experiment. Thus, no metal tools were allowed, and only thin plastic and glassware were used through out the experiment. Table 4.1 shows the apparatus and materials used in the XRF experiment.

	Apparatus and Materials
1	Detector: XR-100T-CZT & PX2T
2	Gamma ray excitation source: Tc-99m (~40.3 mCi)
3	Thin transparency plastic beg
4	Beakers (50 ml)
5	Glass pipette (10 ml & 5ml)
6	Ultra pure water
7	Standard Lead Solution (1000 ppm)
8	Pure copper collimator (thickness: 0.5 cm)

Table 4.1: List of apparatus and materials used in XRF experiment

The beakers and pipette were properly cleaned by means of soaking in the acid water (prepared with nitric acid (65%), AnalR grade acid and ultra pure water) for at least 3 hours to remove all contaminants. Then the beakers and pipette were rinsed with ultra pure water to eliminate the acid before they are used in experiments.

4.4.1 The Preparation of Standard Lead Solutions

Various concentration of lead had been prepared from the Standard Lead Solution (lead (II) nitrate in nitric acid 0.5 mol / l: 998 ± 2 mg/l Pb). The dilution preparations was done using a simple formula:

$$M_1V_1 = M_2V_2$$

For instance, 900 ppm Pb was prepared from a standard 1000 ppm lead solution, which the calculation of the needed stock volume is simply:

$$1000 \text{ ppm} \times V_{Pb} = 900 \text{ ppm} \times 10 \text{ ml}$$

$$\therefore V_{Pb} = 9 \text{ ml}$$

Hence, the volume of 1 ml of ultra pure water needed to top up 9ml of Pb standard solution to obtain the concentration of 900 ppm of Pb. 10 concentration of Pb had been prepared as shown in table 4.2.

Lead Concentration/ ± 2 ppm	**Standard Lead Solution (1000 ppm) / ± 0.1 ml**	**Lead Solution**	
		Lead / ± 0.1 ml	**Pure Water / ± 0.1 ml**
1000	10.0	10.0	0.0
900	9.0	9.0	1.0
800	8.0	8.0	2.0
600	6.0	6.0	4.0
400	4.0	4.0	6.0
200	2.0	2.0	8.0
100	1.0	1.0	9.0
50	0.5	0.5	9.5

Table 4.2: The Preparation of Various Pb Concentrations from 1000 ppm to 100 ppm

4.5 The Experimental Set-up

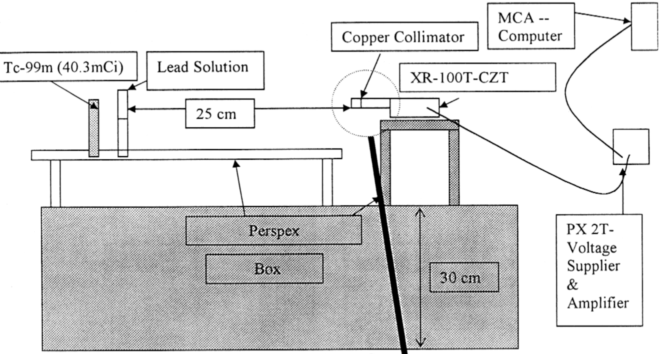


Figure 4.14 (a): The Schematic of Experiment Set-up

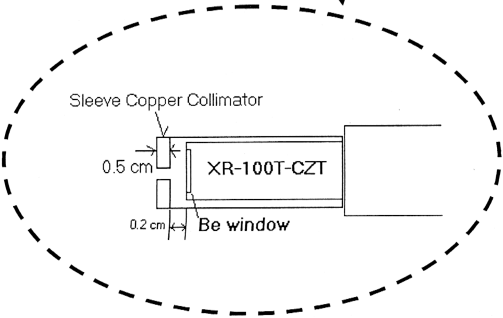


Figure 4.14 (b): The enlarged part from the side view showing the Position of Sleeve Copper Collimator

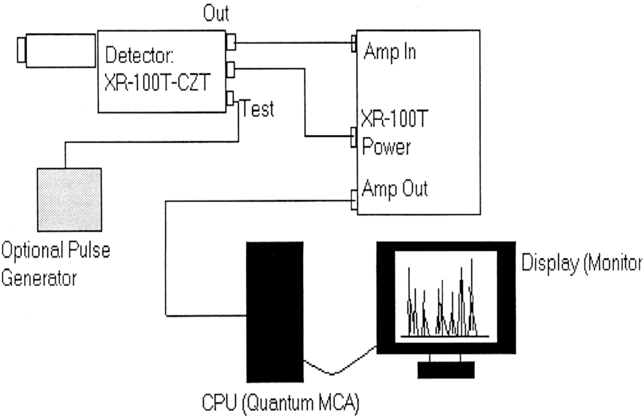


Figure 4.15: Schematic of Connection for the XR-100T-CZT to the PX2CZT and Quantum MCA

Figure 4.14 shows the Perspex holder was used to position the detector and transparency plastic beg (contained the lead solution and excitation source of Tc-99m) at the height of 7 cm and 5 cm from the box respectively. The box, which is 30 cm above the table, plays a vital role to minimize the background effect from any contamination from the surface of table, including the paint, cupboard and PC's casing. The PX 2T which functions as a power supply and shaping amplifier was placed far away from the detector and a tower-cased CPU to avoid any electrical noise interference. The connection of the detector XR-100T-CZT to PX2T (power supplier and amplifier), and Quantum MCA is shown figure 4.14.

A pure copper collimator is designed to sleeve along the arm of detector, as shown in figure 4.13 (b). The thickness of copper collimator is 0.5 cm and is placed 0.2 cm in front of Be window of the detector. The collimator has a very small diameter hole of 5 mm with the purpose of to:

1) Confine the effective area of the detector and exclude regions close to the edge of the crystal where incomplete charge collection can give rise to a deterioration of the peak shape. Therefore, this can improve the long-term stability of the measured efficiency.
2) Reduce the number of scattered photons reaching the detector from the source mount and other nearby materials. (K.Debertin and R.G.Helmer, 1988)

This collimator as well as the detector casing and the surrounding area of Perspex were rinsed with 70% surgical alcohol to remove dirt and any contamination. Throughout the experiment, rubber hand gloves were used to minimize any contamination contributed from handling the source and oil contamination from the fingerprints.

The target sample of lead solution in thin transparency plastic beg was placed in front of the excitation source, Tc-99m, with the distance of 1 cm, while the distance from the detector was 25 cm.

Tc-99m which was used as an excitation source to provide the effective gamma ray of 140.5 keV (half-life ~ 6.02 hours). The spectrum of Tc-99m was shown in figure 4.13.

4.6 System Calibration

The detector system was calibrated by using the gamma ray from Tc-99m, which gives the standard energy at 18.4 and 140.5 keV. The channels of 38-44 and 301-312 for the peaks of Tc-99m were marked and assigned with the corresponding energies of 18.4 keV and 140.5 keV respectively.

The calibration was cross-checked using Am-241 having the energy spectrum of 59.5 keV, 13.9 keV and 17.8 keV corresponding to the channels of 127-133, 27-31 and 36-42 respectively. The spectrums of Tc-99m and Am-241 used in calibration are shown in figure 4.16 (a) and (b) respectively.

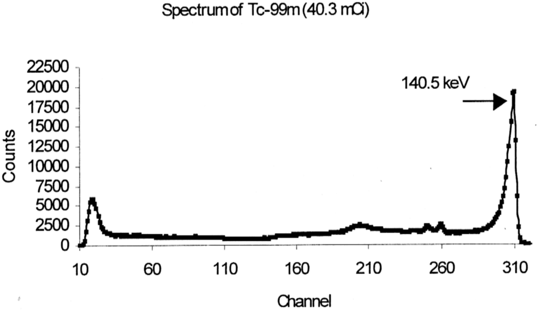


Figure 4.16 (a): The Spectrum of Tc-99m (with the activity of 40.3 mCi)

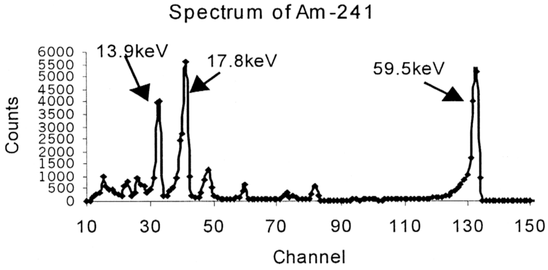


Figure 4.16(b): The Spectrum of Am-241—indicating in the peak energy and the channel number

After the calibration, a piece of lead with the thickness of 2 cm was placed at the position instead of the lead solution. The other settings remain unchanged. The K-lines of lead had been observed, at energy of 72.8 keV, 75 keV and 85 keV as shown in figure 4.17.

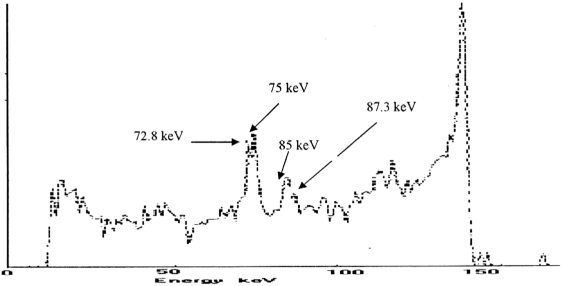


Figure 4.17: Pb and Tc-99m calibration spectrum

The channel numbers, which corresponded to the $K_{\alpha 1}$ and $K_{\alpha 2}$ of lead were determined. The channels were at 160-163 and 165-169 respectively with FWHM of 277 eV and 315 eV respectively. Table 4.3 showed the X-rays peak and energy of Pb.

Peak	Energy (keV)
$K_{\alpha 1}$	74.9
$K_{\alpha 2}$	72.8
$K_{\beta 1}$	84.9
$K_{\beta 2}$	87.3
$K_{\beta 3}$	84.5

Table 4.3: The K-lines and correspond energy of Pb

4.7 Experimental Procedure

The detector had been set, using the following parameters: the Amplifier Gain of PX2T was fixed at 0.5, while both the group gain and conventional gain of Quantum MCA were fixed at 1024 respectively. The systems setting are fixed throughout the whole experiment. The following procedures were taken before each experiment was carried out:-

(i) The detector system was switched on to warm up. This would take less than 2 minutes.

(ii) The position of each component was checked and fixed at stated in 3.5.

(iii) The activity of Tc-99m was fixed in the range of ~ 40 to ~40.5 mCi throughout the whole experiment.

(iv) The measurement time of each sample was fixed at 400 seconds.

(v) Each sample solution was measured at least three times. These repeated measurements could reduce the system error and reproducibility test.

(vi) Blank samples (ultra pure water) were prepared. It was used for normalization purposes and for background detection. The measurement was made prior to the counting of each sample.

(vii) The target sample was started with the known concentration of Pb, i.e. 1000 ppm and then followed by 900 ppm, 800 ppm, 600 ppm, 400 ppm, 200 ppm, 100 ppm and 50 ppm. (Chea-Beng, Lee et.al., 2000)

4.8 The Experimental Preparation for Induced Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES)

Human teeth samples had been collected; merely from adult donors of two areas (Klang Valley and Malacca). The samples from Klang Valley, covered the areas of Puchong, Old Klang, OUG, P.J., Kuchai, Kg.Medan and Subang; while the samples from Malacca, were mainly from Bachang, Peringgit Jaya, Merlimau, Bandar Hilir, Malim Jaya, Bukit Beruang, Bukit Rambai, Batu Berendam and Air Keroh as well as from Semabok.

Prior to the teeth extraction, personnel information such as type of work, place of resident, etc had been recorded.

4.9 Treatment for Teeth Samples from Adult Donors: Preparing Teeth Samples

Immediately after being extracted, the teeth were treated with Clorox solution for approximately 5 minutes. This step is taken to kill any germs that may still be presented in the extracted teeth and in the blood as to avert any possible biological hazard. The extracted teeth may have some gum tissues and caries on them; so by washing them in the Clorox solution could ease the chemical cleaning stage later on. The teeth were then put into the labeled plastic test tubes. The solid teeth are changed to liquid by digesting into solution form before being studied using ICP-AES. Below are the steps taken in ICP technique:

- The teeth was rinsed with ultra pure water to wash away the Clorox solution and each tooth was then put into a 25 ml HNO_3 (40%) solution for about 10 minutes to exclude caries and any organic compound such as dead tissues from the tooth's surfaces.
- Then the tooth was treated with ultra pure water again to terminate any further reactions and clean away some tiny tissues and unsought material.
- Then the tooth was douched with acetone (Anal R Grade) to quickly dry away the ultra pure water. The tooth was put into a clean-labeled beaker and baked in the oven at 133^0 C for 5 minutes to ensure the tooth was completely dried. Figure 4.18 shows the picture of the oven (1500 Furnace).
- After the beaker and tooth have cooled down, the tooth mass was recorded by using an electronic balance. First, both of them were weighed, then the beaker was weighed again after the tooth was transferred to a clean labeled test tube.

- 25 ml HNO_3 (10%) was added into the test tube, which contained the clean tooth and brought to 80°C for 1 hour on a hot plate, then left over night and brought again to 80°C for another 1 hour to make sure the digestion was fully completed. Figure 4.19 shows the test tubes in a big beaker (500 ml) on a hot plate.
- When the test tube had cooled down to room temperature, the sample was filtered with Whatman 540 Hardened Ashless filter paper into a 50 ml standard volumetric flask and topped up to the mark with ultra pure water. Figure 4.20 shows the filtered teeth samples in 50 ml standard volumetric flasks.
- A blank was prepared following similar steps but without tooth.

(L.T.Chew et.al., 2000)

This thorough cleaning procedure would not remove some of these trace elements, though they tend to accumulate near the surface layers of teeth. The acid used in this procedure, HNO_3, is not able to remove or has reaction with enamel of tooth in 10 minutes time. Therefore, none of these elements will be removed from teeth samples.

4.10 ICP-AES Calibration

The concentrations of Pb, Cd, Cu, Mg, Zn and Ca were evaluated using an ICP-AES system, as shown in figure 4.21 (Varian ICP). Before making measurements, the system was first aspirated with ultra pure water to clean the system. Instrument calibration was carried out by employing standard solutions, which were prepared for two different concentrations for each element. The desired concentrations were obtained by diluting the appropriate volume from 1000 ppm stock solution into a single 50 ml volumetric flask. Each stock solution was in spectrosoL grade. Table 4.4 shows the standard solutions with the known concentrations for each element.

Element	Concentration (ppm) in 50 ml volume flask	
Pb	50	5
Mg	5	50
Zn	500	100
Cu	50	10
Cd	0.1	0.006
Ca	800	100

Table 4.4: Preparation of the Standard Solution with the known Concentration for Each Sample

The following emitted wavelengths were used for each element: Pb (261.418nm), Mg (279.553 nm), Zn (213.856 nm), Cu (324.754 nm), Cd (228.802 nm) and Ca (317.933 nm). The duration of measurement for each sample was of the order of 10 seconds.

The wavelength calibration procedure was done in two parts; a coarse adjustment followed by a fine adjustment. Before this, the system was aspirated with the blank solution. For the coarse adjustment, the system was allowed to aspirate in the individual wavecal solution and the data processed. The wavelengths and maximum intensities were corrected in the fine adjustment part.

4.11 The Summarized Procedure of ICP Operation

As the accuracy of ICP determination depends on the procedures followed during the experiment, the following steps were taken:

- First turn on the room fan, ventilator and air condition
- Turn on the vacuum pump

- Turn on power supply
- Turn on water cooler (check the flow rate, at 6 LPM (liter per minute and temperature at 20° C)
- Open the Argon cylinder from gas room and put the gauge at 60-psi (consumption is ~ 25 bar per hour)
- Turn on ICP
- Turn on computer and printer
- Arrange tubing of pump and on the pump, put some deionized water in the container
- Turn RF switch on and turn on the torch

4.12 Experimental Procedure

Once the ICP system had been calibrated, the measurement of teeth samples would be rather simple. First, the sample number was entered and the sample capillary tube was cleaned by dipping it in the deionized water.

The system analyzed the interested elements in sequence. Each measurement of sample took about 2 minutes with 10 seconds countdown before measurement. The measurements were continued continuous until all the elements interested were measured. As a precaution, the system was recalibrated after the certain samples had been measured (~ 40 samples) to ensure accuracy.

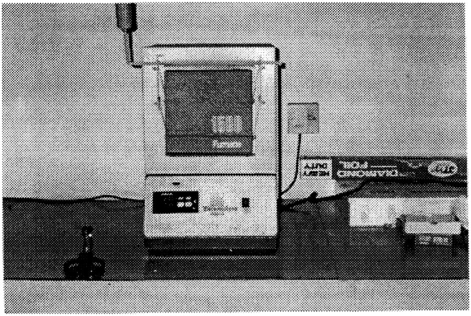


Figure 4.18: The Furnace use to dry Teeth Samples

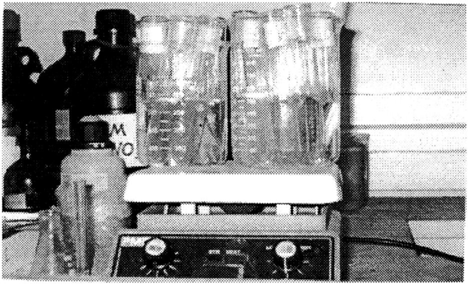


Figure 4.19: The Teeth Samples Digested in Test Tubes on a Hot Plate

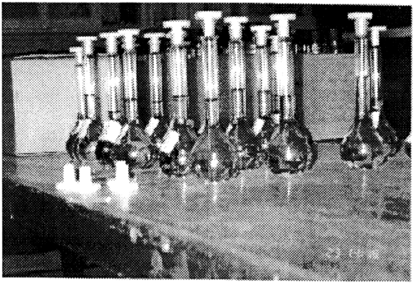


Figure 4.20: Filtered Teeth Samples in 50ml Standard Volumetric Flask

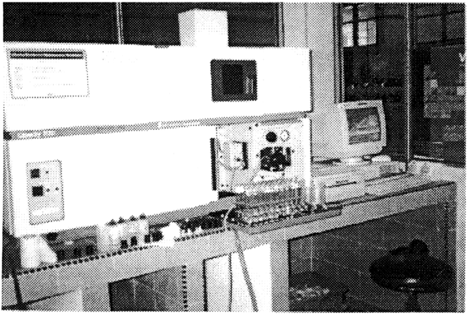


Figure 4.21: Varian ICP

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AMPTEK User's Manual.

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