Program setup and parameter dependences of GUPIXWIN-calculated trace element concentration measured by PIXE analysis

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Abstract. In our practice of applying GUPIXWIN software in calculations of the trace element concentrations in biosamples measured by particle induced X-ray emission (PIXE) technique, we found that the calculated concentration data critically depended on the program setup and experimental parameters input to the software. Dependences on some important setups and parameters such as the solution types (i.e. Trace element solution in a known matrix and Iterative matrix element solution), matrix composition, detector parameters (e.g. window thickness), sample structure, normalization, etc., which are sometimes easily not paid with intensive attention by users, are analyzed and discussed utilizing our PIXE spectra of some biosamples. We found that most parameters or program setups were critical to affect the calculated results, but some such as normalization was not. We also found that if only relative trace element concentrations instead of their absolute values are concerned, different program setup solutions resulted in similar data. We conclude that meticulous care must be taken in selection of the experimental parameters and setup conditions in using GUPIXWIN to make correct calculations of the trace element concentrations in biological or organic samples.

1. Introduction
The particle induced X-ray emission (PIXE) is a well-known technique as a trace element analysis method using a few MeV ion beam. This technique can be applied to various fields such as biology, medicine, environment, earth sciences, art and archaeology in addition to materials science [1]. In PIXE analysis of materials, a high-energy (generally > MeV) proton or heavy ion beam from an accelerator bombards a target material and interacts with the inner shell electrons of target atoms, resulting in knocking out electrons from inner shells. To restore equilibrium of energy of the atom, outer shell electrons having higher energy levels fall to refill the inner shell vacancies. Since the inner shell has a lower energy level, the excess energy is emitted in the form of X-ray. The released X-ray has a characteristic wavelength or energy due to the specific arrangement of electrons in each atomic species. So, we can analyze the elemental composition in the sample by detecting the characteristic X-rays. Furthermore, the amount or concentration of the elements in the sample can be determined from the...
characteristic X-ray intensities in the PIXE spectrum which is the particle-induced characteristic X-ray intensity as a function of the X-ray energy. For quantitative determinations of the elements and their concentrations from the spectrum, one must use the software package of GUPIXWIN [2,3]. The information input to GUPIXWIN is a fairly complicated systematic engineering job as highlighted in table 1. Dozens of conditions and parameters have to be correctly set up and input when one starts the program for calculations. Any slightly small difference in the input information might lead to observable differences in results. In our practice of applying GUPIXWIN software in calculations of the trace element concentrations in biosamples measured by PIXE, we found that the calculated concentration data critically depended on the program setup and experimental parameters input to the software. Dependences on some important setups and parameters such as the solution types (i.e. Trace element solution in a known matrix and Iterative matrix element solution), matrix composition, detector parameters (e.g. window thickness), sample structure, normalization, etc. are sometimes easily not paid with intensive attention by users so that ridiculous errors may occur. In this presentation, we make some discussions about the dependencies based on examples of our PIXE-analysis of biosamples.

| Table 1. GUPIXWIN information input structures. |
|-----------------------------------------------|
| **Primary requirements** | **Secondary requirements** | **Experimental conditions** | **Parameters or remarks** |
| Setup | **Trace or Matrix** | TESinKM (Trace element solution in a known matrix) | Used for the trace elements to be determined in a known matrix (in which there are invisibles) |
| | IMES (Iterative matrix element solution) | Used for all elements of the sample to be determined |
| | Angles | Beam, X-ray |
| | Beam parameters | Ion, Energy, Beam charge (Q) |
| | Detector parameters | Detector selection (from detector files): e.g. window thickness |
| | Energy dependence | Constant or dependent |
| | H-value | |
| Sample | **Sample structure** | Thin | Final ion energy = initial energy |
| | | Thick | Final ion energy = 0 |
| | | Intermediate | Final ion energy (to be calculated) |
| | | Layered | Layer information |
| Trace element solution (TESinKM) | Define matrix elements | Input matrix element composition |
| Define trace elements | Input trace elements |
| Matrix element solution (IMES) | Define fit elements | Input the elements to be fitted |
| Define invisible components | Input invisible elements or compounds |
| Fit | Spectrum details | Region of fit | The channel number region |
| | | Stopping criterion | E.g. maximum iterations |
| | | Calibration parameters | A1, A2, A3, A4, A5 |
| | | Pile-ups | Number of pile-ups |
| | | Digital background filter | Filter options |

2. Experiments
The PIXE analysis was carried out using 2-MeV proton beams at the MeV normal beam or capillary microbeam facility, Chiang Mai University (CMU) [4]. The technical details of CMU PIXE setup have been described elsewhere [5]. In brief, the X-ray detector was a Si(Li) detector with an energy resolution of 150 eV and the angle between the detector and the sample surface normal is 60°. The detector had an active area of 30 mm² with a 25 µm thick beryllium window, leading to the H-value of 0.002721. The samples were various biological tissues which were local longan leaves, herb (Peperomia pellucida) leaves and human cardiac muscle. The concentration of each element proportional to the intensity of the X-ray emitted from the element was determined by using the software package of GUPIXWIN (V2.1) [2]. Since the biosamples are dominantly composed of light elements such as hydrogen, carbon, nitrogen and oxygen which are invisible by PIXE, in running GUPIXWIN, the Trace element solution in a known matrix (TESinKM) must be used, instead of the Iterative matrix element solution (IMES) (without invisible information). TESinKM deals with a sample whose matrix compositions, including the PIXE-invisibles, are known but trace element concentrations are unknown and thus to be determined. IMES deals with a sample whose elements are all considered to be the matrix elements but with unknown concentrations which are to be determined by program iteration to fit a pre-assumed spectrum to the measured spectrum. IMES is generally used for analyzing normal solids in which the dominant elements are visible, while TESinKM is more suitable to analyzing trace elements in bio- and organic materials in which the dominant elements are invisible. As biosamples normally have very complicated chemical compositions, the chemical compositions of a known matrix mostly have to be input by assumptions, which however would not substantially alter the trace element concentrations calculated by the software. In our most analyses, the plant tissue matrix is assumed to be cellulose, which is the dominant component in the plant cell wall, in C₆H₁₂O₆ with the mass density of about 1 g/cm³, and the muscle matrix is assumed to be a muscle equivalent in H₁₀C₁₆N₄O₇₁ with the mass density of about 1.1 g/cm³ (from the SRIM database [6]). For comparisons, in running GUPIXWIN, both TESINKM and IMES solutions, varied detector window thickness of 25 µm and 75 µm, and varied the Sample Structure of thin film, thick and intermediate thickness were tested.

3. Results and discussion
Longan leaf samples were originally sprayed by farmers with chemical fertilizer (18-8-8 of N-P-K) as well as sometimes chemical pesticide. The thickness of the leaves was about a few hundreds of micrometers and hence the sample structure in the program should be a thick sample. From the PIXE spectra of the longan leaf sample, totally six trace elements, Al, S, Cl, K, Ca and Fe were identified and their concentrations were calculated using different solutions under the condition of different detector window thicknesses as shown in table 2 for an example.

The herb plant was cultured in nutrient solution before PIXE analysis. The thickness of the leaves was around 100 µm and hence the sample structure in the program was set as an intermediate sample. From the PIXE spectra of the leaf sample, selectively six trace elements, Al, S, Cl, K, Ca and Fe, were detected and identified from their Kα lines with the concentrations in dominance. The trace element concentrations were calculated from the spectrum for different detector parameters, as shown in table 3 for an example.

Myocardial samples were collected from dead body hearts. The thickness of the samples was about 1 mm and hence they were thick samples. From the PIXE spectra, seven trace elements, i.e. Mg, Si, P, S, Ar, K and Mn, were detected and identified. The PIXE-measured/GUPIXWIN-calculated trace element concentration data for different detector parameters are shown in table 4 as an example.

From the results shown above, we observed some differences for different conditions. The concentrations calculated by TESinKM are considerably lower than those by IMES. This is very reasonable, because TESinKM already includes a major concentration background of the known matrix elements set up by this solution and the trace element concentrations obtained are only minors relative to this background, whereas in IMES (without the invisible information) the concentrations of the “traces” which are all taken as the major matrix elements are relative to themselves. Note that quite lots
Table 2. An example of GUPIXWIN-calculated concentrations (in ppm) of PIXE-identified elements in a longan leaf sample under varied conditions. →: increasing from left to right. (a) Using the Trace element solution in a known matrix (TESinKM). (b) Using the Iterative matrix element solution (IMES).

(a)

| Sample structure | Detector window thickness (µm) | Elements (Z →, X-ray energy →) |
|------------------|-------------------------------|--------------------------------|
|                  | Al   | S    | Cl   | K    | Ca   | Fe   |
| Thin             | 25   | 3    | 26   | 45   | 1854 | 2999 | 97   |
|                  | 75   | 18   | 41   | 61   | 2149 | 3328 | 100  |
| Thick            | 25   | 7    | 20   | 28   | 794  | 1156 | 32   |
|                  | 75   | 44   | 32   | 37   | 921  | 1285 | 33   |
| Intermediate     | 25   | 8    | 21   | 30   | 947  | 1424 | 42   |
|                  | 75   | 36   | 34   | 42   | 1104 | 1580 | 42   |

(b)

| Sample structure | Detector window thickness (µm) | Elements (Z →, X-ray energy →) |
|------------------|-------------------------------|--------------------------------|
|                  | Al   | S    | Cl   | K    | Ca   | Fe   |
| Thin             | 25   | 103  | 836  | 1438 | 59436| 96103| 3113 |
|                  | 75   | 569  | 1305 | 1954 | 68873| 106677|3199 |
| Thick            | 25   | 3072 | 7416 | 9592 | 234717|725136|20039|
|                  | 75   | 11740| 10508| 12168| 241837|707386|16438|
| Intermediate     | 25   | 2965 | 7193 | 9388 | 241323|719243|19832|
|                  | 75   | 11387| 10220| 11934| 248103|702059|16320|

Table 3. An example of GUPIXWIN-calculated concentrations (in ppm) of PIXE-identified elements in a herb leaf sample under varied conditions. (a) Using the Trace element solution in a known matrix (TESinKM). (b) Using the Iterative matrix element solution (IMES).

(a)

| Sample structure | Detector window thickness (µm) | Elements (Z →, X-ray energy →) |
|------------------|-------------------------------|--------------------------------|
|                  | Al   | S    | Cl   | K    | Ca   | Fe   |
| Thin             | 25   | 2    | 12   | 4    | 503  | 1457 | 286  |
|                  | 75   | 9    | 18   | 5    | 580  | 1610 | 290  |
| Thick            | 25   | 4    | 9    | 2    | 215  | 565  | 97   |
|                  | 75   | 21   | 13   | 3    | 248  | 626  | 99   |
| Intermediate     | 25   | 4    | 9    | 2    | 258  | 696  | 123  |
|                  | 75   | 21   | 14   | 3    | 299  | 772  | 125  |

(b)

| Sample structure | Detector window thickness (µm) | Elements (Z →, X-ray energy →) |
|------------------|-------------------------------|--------------------------------|
|                  | Al   | S    | Cl   | K    | Ca   | Fe   |
| Thin             | 25   | 40   | 374  | 130  | 15656| 45413| 8890 |
|                  | 75   | 221  | 578  | 171  | 18100| 50349| 9055 |
| Thick            | 25   | 3196 | 8952 | 2134 | 155331|707517|122591|
|                  | 75   | 14994| 12178| 2494 | 160262|700532|109232|
| Intermediate     | 25   | 3100 | 8717 | 2098 | 158830|704533|122412|
|                  | 75   | 14573| 11878| 2455 | 163733|698053|108946|
Table 4. An example of GUPIXWIN-calculated concentrations (in ppm) of PIXE-identified elements in a cardiac muscle sample under varied conditions. (a) Using the Trace element solution in a known matrix (TESinKM). (b) Using the Iterative matrix element solution (IMES).

(a) Sample structure | Detector window thickness (µm) | Elements (Z →, X-ray energy →) |
|---------------------|-----------------------------|-------------------------------|
|                     | Mg  | Si  | P   | S   | Ar  | K   | Mn  |
| Thin                | 25  | 683 | 14  | 75  | 95  | 403 | 89  | 220 |
|                     | 75  | 11701 | 25 | 134 | 131 | 483 | 99  | 216 |
| Thick               | 25  | 3059 | 15  | 82  | 70  | 206 | 37  | 63  |
|                     | 75  | 52906 | 59 | 169 | 108 | 251 | 42  | 63  |
| Intermediate        | 25  | 3059 | 15  | 83  | 72  | 228 | 42  | 79  |
|                     | 75  | 52922 | 43 | 164 | 112 | 280 | 49  | 81  |

(b) Sample structure | Detector window thickness (µm) | Elements (Z →, X-ray energy →) |
|---------------------|-----------------------------|-------------------------------|
|                     | Mg  | Si  | P   | S   | Ar  | K   | Mn  |
| Thin                | 25  | 674 | 16  | 73  | 79  | 380 | 87  | 199 |
|                     | 75  | 11662 | 46 | 145 | 124 | 468 | 101 | 203 |
| Thick               | 25  | 569334 | 15696 | 69842 | 64198 | 199806 | 45568 | 36099 |
|                     | 75  | 891071 | 9537 | 28248 | 18798 | 41346 | 6699 | 4897 |
| Intermediate        | 25  | 569285 | 15643 | 69465 | 63791 | 198508 | 45170 | 38618 |
|                     | 75  | 890480 | 9430 | 28074 | 18692 | 41203 | 6710 | 5487 |

Table 5. An example of trace element concentrations (in ppm) calculated using TESinKM and IMES with the invisible information (cellulose: C_{6}H_{12}O_{6}) input from a longan leaf sample.

| Solution          | Al  | S   | Cl  | K   | Ca  | Fe  |
|-------------------|-----|-----|-----|-----|-----|-----|
| TESinKM           | 8.4 | 1.4 | 7.8 | 21.1 | 35.9 | 100.1 |
| IMES including invisibles | 8.4 | 1.3 | 7.5 | 20.8 | 35.7 | 101.5 |

of calculations performed for biosamples before only use IMES without giving the invisible information for trace element concentrations as a simple way and hence errors should be inevitable. However, when the TESinKM and IMES concentrations were normalized for relative concentrations, we found the normalized relative concentrations fairly similar between the two types of solutions, as demonstrated in figure 1, where the data came from three different PIXE analyzed sample locations to show the general trend. Note that the trace element concentrations calculated by TESinKM are absolute, as all concentrations include those of the matrix elements, but if only the trace elements are considered, their relative concentrations could be calculated from normalization of their concentrations only, while the IMES-calculated trace element concentrations, if without invisible information input, are only relative concentrations of the trace elements. Moreover, when using IMES if the invisible information was input, the trace concentrations calculated from both TESinKM and IMES were almost the same (table 5).
Figure 1. Examples of the normalized relative concentrations (the vertical axis) of all trace elements (the horizontal axis) in the longan leaf sample from 3 different analyzed positions (as noted on the top of each figure), calculated by using IMES and TESinKM, respectively, with the trendlines of 5-order polynomial fitting. The numbers at the plot tops only designate the positions analyzed on the leaf.

Therefore, in order to obtain the absolute trace element concentrations, one can apply either TESinKM or IMES with invisible information input. While the differences in the concentrations between thin and thick samples are obvious, some differences between thick and intermediate samples can be seen anyway existing. While the analyzing ions totally lose their energy in the thick sample, they lose the energy partly in the intermediate sample depending on the sample thickness, and hence induced X-ray mission intensities are not the same for both cases. Note that lots of calculations performed before only adopted the thick sample structure as an easy way for all types of samples and thus small errors could occur. Comparing using different detector window thicknesses, we found that in most cases, especially for lighter elements which should have lower X-ray energy, the thinner window gave lower concentrations than the thicker window. As thicker windows more attenuate the lower energy X-rays so that the lower energy X-rays that pass the window and are detected could be over sensitized, the lower energy X-rays emitted from the lower Z elements are exaggerated. Note that almost all calculations carried out before only used the thicker window (75 µm) due to a simple fact that no data for the thinner window in the detector file in the program and consequently mistakes in the calculated concentrations could happen. In this new test, we added a new detector in the detector file by editing the file with inputting the 25 µm thickness for the detector window.
4. Conclusion
We tested some different input conditions or parameters in the GUPIXWIN program to calculate trace element concentrations of PIXE-analyzed biosamples for investigations on relevant effects on the final results. The conditions or parameters included different solutions in the program Setup, sample structures, detector window thickness and concentration normalization, which could be easily overlooked by PIXE analysis novices. We emphasize that meticulous care must be taken in selection of the experimental parameters and setup conditions in using GUPIXWIN appropriate to the real applied conditions to make correct calculations of the trace element concentrations in biological or organic samples.

Acknowledgement
The work has been supported by the Development and Promotion of Science and Technology Talents Project of Thailand, the Chiang Mai University, and the Thailand Center of Excellence in Physics. We wish to thank Chome Thongleurm for the technical assistance in the accelerator.

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