Brazilian Journal of Analytical Chemistry
2023, Volume 10, Issue 38, pp 13-17
doi: 10.30744/brjac.2179-3425.letter-hudsoncarvalho.N38

BrJAC

*LETTER*

# Past, Present, and Future of X-ray Fluorescence Chemical Imaging

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Elemental chemical images reveal how the elements are distributed in a sample. While it may sound useless for a homogeneous solution, it is crucial for understanding properties of heterogeneous systems, such as a rock[1], a cereal grain[2], or a painting[3]. In such cases, revealing the chemical composition of parts of the sample might reveal the distribution of minerals, nutrients, or toxic elements.

All strategies for measuring the spatial distribution of elements require a probe, whose size will define the lateral resolution of the image, and a detection system. Some techniques are destructive while others preserve the specimen; this latter feature is of special importance for rare and mass-limited samples or in the case of *in vivo* analysis. Laser ablation coupled to mass spectrometry or optical emission spectrometry, laser induced break down spectrometry, and microprobe X-ray fluorescence spectrometry are some of the most common laboratory techniques employed in chemical imaging. The latter technique is discussed below.

### *What is X-ray fluorescence?*

X-ray fluorescence spectrometry (XRF) is a well-established technique used to identify and quantify elements, usually with atomic number above sodium. XRF equipment is commonly found in research & routine laboratories, online sorting, field prospection of minerals, and classification of metal scraps. A striking advantage of XRF over other techniques regards the ability of carrying out a direct analysis, *i.e.*, samples can be probed without matrix digestion. This is a key feature for chemical imaging.

X-rays were first reported by W.C. Roentgen in 1895. They are part of the electromagnetic spectrum, arguably defined between 100 eV to 100 keV. X-ray fluorescence, *i.e.*, the emission of X-rays by atoms, is one of the possible relaxation processes occurring upon the formation of a core-hole, which can be produced by the ejection of a core electron through momentum transfer either from a photon or particle. Once this core-hole is established, the electronic configuration is disturbed, and the atom passes into an excited state; it comes back to ground state when another electron from a higher level makes a transition filling the core-hole. The energy difference between these two levels may entail a relaxation process with the emission of a characteristic photon, which is called X-ray fluorescence. The energy of the characteristic photon depends on the atomic number, which allows one to identify the atom; this fact was first reported by C.G. Barkla in 1908. By 1913, H. Moseley figured out that the square root of the X-ray frequency held a linear relationship with the integer atomic number; this structure led him to state the existence of three unknown elements with atomic numbers 43 (technetium), 61 (promethium), and 75 (rhenium). The first accurate X-ray spectrometers for chemical analysis started being built by K.M.G. Siegbahn in 1916.

### *Analytical facilities for X-rays fluorescence*

The excitation of atoms can be accomplished by X-rays sources, electron beams, or proton beams. These three types of sources can be employed for chemical imaging. Proton beams offer limits of detection

**Cite:** Carvalho, H. W. P. Past, Present, and Future of X-ray Fluorescence Chemical Imaging. *Braz. J. Anal. Chem.,* 2023, *10* (38), pp 13-17. http://dx.doi.org/10.30744/brjac.2179-3425.letter-hudsoncarvalho.N38

(LOD) on the order of a few μg $kg^{-1}$; nevertheless, they require complex and expensive infrastructure. Conversely, electron microscopes are widely available, but they probe only the first few micrometers of the sample surface, and the spectral background leads to LOD on the order of g $kg^{-1}$. A good compromise between accessibility and LOD are met by the X-ray excitation sources; LOD are in the order of a few mg $kg^{-1}$ for laboratory sources and a few μg $kg^{-1}$ for synchrotron sources.

Another instrumental parameter pivotal for the sensitivity is the type of detector. There are two main groups of detectors: wavelength dispersive detectors offer lower LOD and avoid spectral interferences, while energy dispersive detectors are simpler, cheaper, and faster, with LOD one order of magnitude higher. The latter is the most commonly employed in chemical imaging, since it can detect the elements simultaneously under dwell times varying from μs to s per pixel.

### *Strategies for obtaining chemical images*

A usual approach for producing a chemical image relies on scanning the sample with an incoming excitation beam. The lateral resolution is a function of sample thickness, beam width, and step size. The detection of the outgoing XRF is accomplished by an energy dispersive X-ray detector. Some wavelength dispersive XRF spectrometers also offer the possibility of sample mapping under sequential detection; however, this is rarely reported. When the samples move relative to the beam, the imaging strategy is called scanning X-ray fluorescence microscopy (SXFM) or X-ray fluorescence microanalysis (μ-XRF).

The main challenge consists of producing a sufficiently small excitation beam. Unlike electrons or protons that can be focused by magnetic lenses or visible light that presents an appreciable diffraction index, the X-ray trajectory is hardly changed by interaction with materials. Thus, the first X-ray chemical images were obtained using pinholes yielding sub mm excitation beams. Nevertheless, pinholes discard most of the X-rays, and only a fraction of the power is dedicated to sample excitation. More sophisticated optical elements, such as capillaries, Kirkpatrick-Baez mirrors, or Fresnel zone plates, are capable of producing nm high density flux X-ray beams. Capillaries are commonly found in laboratory equipment, while the mirrors and zone plates are found in synchrotron beamlines.

Volume images, *i.e.*, 3D, can be obtained by XRF tomography, where the sample is rotated during the measurement. In addition to X-ray transmission, the X-ray fluorescence data is also recorded. This yields a sequence of segments as a function of the sample angle called sinograms, which can be reconstructed to form a back projection that corresponds to a slice or a volume of the sample. Although powerful, 3D images take much longer to record than 2D images, increasing the cost and subjecting the sample to a larger dose of radiation. One should not forget that X-ray escape depth depends on the element, so the sample size is limited from hundreds of μm to few mm.

### *Laboratory & synchrotron scanning XRF microscopes*

Homemade equipment (Figure 1A) is an alternative to commercial scanning XRF microscopes, which costs a few hundreds of US dollars. In addition to electricity and power backup, they require little preventive maintenance and few consumables, such as polymeric thin films employed in sample holders. Some commercial equipment offers the option of vacuum or helium atmosphere, which increases the sensitivity for atomic numbers below titanium, and removal of the argon K lines, which may improve the LOD for chlorine, potassium, palladium, silver, and cadmium. They are usually furnished with 50 W X-ray tubes and capillaries, providing an X-ray beam width on the order of a few to tens of μm. The LOD for practical integration times, *i.e.*, below 5 s per pixel, is on the order of a few mg $mg^{-1}$ for organic matrices, such as plants. That said, the costs for acquiring and keeping a scanning XRF microscope is far below those of a laser ablation induced coupled plasm mass spectrometry, for example.

On the other hand, synchrotron beamlines offer higher lateral resolution, *i.e.*, smaller beams, higher X-ray flux density, polychromatic or monochromatic excitation, and usually a large area detector, which increases the solid angle of photon capture. All of this is translated into lower LOD, higher analytical frequency, and a myriad of experimental possibilities. The main drawback of these facilities might be the

limited access for external users, which usually is granted upon a highly competitive process of proposal evaluation. It means that not all research groups would have access to beamtime, and this access occurs, in the best scenario, a few times per year. Thus, the margin for error during an experiment is narrow. Synchrotron facilities, such as the Brazilian Synchrotron Light Laboratory (LNLS), greatly improved user experience by offering the possibility of fast-track application, which allows users to run quick experiments throughout the year. The Brazilian community can count on an excellent facility, the Sirius synchrotron, which was designed to offer high brilliance and small coherent beam spots (https://www.lnls.cnpem.br/facilities/carnauba/).

***Examples of applications***

Selenium is a nutrient for humans; however, most people have diets deficient in this nutrient, which ends up causing diseases. We have recently shown the spatial distribution of elements in Brazil nuts, which are reputed for their appreciable selenium content. Figure 1B shows that the spatial distribution of selenium in this nut is not homogenous. The images revealed that selenium accumulates more on the basal position, which connects the nut to the fruit. Furthermore, the chemical images also revealed the presence of bromine in the samples, which was not expected at the beginning of the study.[4]

Under controlled X-ray dose, XRF chemical images can monitor dynamic phenomena such as those occurring under *in vivo* conditions. Figure 1C shows the uptake of zinc fertilizer by a soybean leaf; the changing pattern indicates that zinc is being transported through the leaf. Until a few years ago, this type of study depended exclusively on radioisotopes that, in spite of low LOD, are more difficult to obtain and handle. Hence, XRF images can support the development of more efficient fertilizers.[5]

Animals can also be mapped; Figure 1D shows the spatial distribution of zinc in *Daphnia magna* exposed to ZnO nanoparticles. The images express the concentration of zinc as mass/area. Contrary to the expected, the study did not detect nanoparticle depuration over time, showing the persistence of the nanomaterial in the model organism.[6]

***Perspectives for XRF chemical imaging***

Static images, such as those conventionally recorded for a polished mineral sample, are similar to analogous photographs; they reflect an instant of something that was occurring. To describe how a system works and create a model able to predict its behavior, a keen scientist would have to assemble a puzzle composed of many of those pictures. Observing such a system under working conditions would make this task much easier and likely more accurate. Hence, we believe that the applications might pressure equipment developers for faster and larger detectors able to decrease both LOD and X-ray exposure times, making measurements more feasible under dynamic conditions, especially for living organisms.

Being minimally invasive and virtually non-destructive is definitely a striking advantage of XRF. Elemental chemical images can be combined with other imaging techniques, such as visible-near infrared reflectance, to display the spatial distribution of organic molecules. For example, in plant science, plant electric potentials and transpiration can be recorded alongside imaging. Hence, the combination of imaging techniques is also a trend.

To reduce the pressure on synchrotron beamlines, we should also consider multiuser, benchtop or laboratory, scanning X-ray fluorescence spectrometers as a potential tool. These facilities can be regarded as complementary. Beyond solving several scientific cases, lab equipment can offer users the opportunity to carry out preliminary studies and more assertively select the samples that deserve precious synchrotron beamtime.

Other recent advancements regard the establishment of full-field X-ray fluorescence imaging. This promising strategy might dramatically reduce the time for obtaining the image, since it does not require scanning the sample. In a full-field setup, the area of interest is completely illuminated by an incoming beam, while the X-ray fluorescence is collected by a pinhole collimator or by a capillary system that guides it to the position and energy sensitive detector that will produce the elemental image.[4]

Finally, as equipment is improving in efficiency and becoming more user friendly, scientists from different fields of knowledge might start employing X-ray fluorescence as an imaging technique. Quantification remains a challenge due to matrix effects; since samples are not homogenous, they are not straightforward to correct. This latter issue might be addressed by multivariate strategies of data analysis.

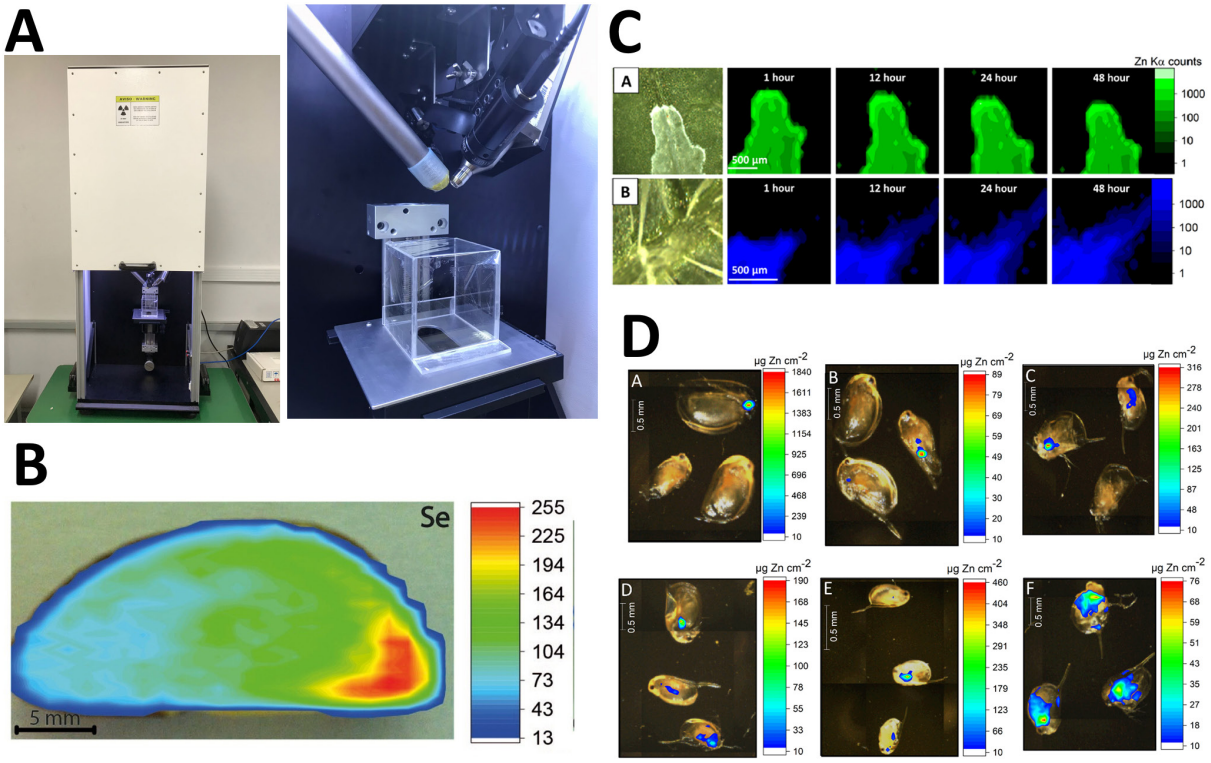


**Figure 1.** (A) Home-made X-ray fluorescence spectrometer for microanalysis, the 2 mm X-ray beam is shaped by a collimator. (B) Elemental map showing the heterogeneous distribution of selenium in Brazilian nut, reprinted from Journal of Food Composition and Analysis, 106, E.C. Silva Jr, N.M. Duran, J.H.L. Lessa, P.G. Ribeiro, L.H.O. Wadt, K.E. Silva, R.M.B. Lima, K.D. Batista, M.C. Guedes, R.C. Oliveira Jr., H.W.P. Carvalho, A.R. Reis, G. Lopes, L.R.G. Guilherme, Unravelling the accumulation and localization of selenium and barium in Brazil nuts using spectro analytical techniques, Article 104329, Copyright (2022), with permission from Elsevier. (C) Chemical imaging showing the spatial distribution of zinc in a soybean leaf, reprinted with permission from Journal of Agricultural and Food Chemistry, 67, 12172-12181, *In Vivo* evaluation of Zn foliar uptake and transport in soybean using X-ray absorption and fluorescence spectroscopy, M.H.F. Gomes, B.A. Machado, E.S. Rodrigues, G.S. Montanha, M.L. Rossi, R. Otto, F.S. Linhares, H.W.P. Carvalho. Copyright 2019 American Chemical Society. (D) Spatial distribution of zinc in the body of *D. magna* exposed to zinc oxide nanoparticles, reprinted from Science of The Total Environment, 2022, 821, J.R. Santos-Rasera, R.T.R. Monteiro, H.W.P. Carvalho, Investigation of acute toxicity, accumulation, and depuration of ZnO nanoparticles in *Daphnia magna*, Article 153307, Copyright (2022), with permission from Elsevier.

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**Hudson Wallace Pereira de Carvalho**, Associate Professor, has been at the Centre of Nuclear Energy in Agriculture of the University of Sao Paulo (CENA-USP) since 2015. His doctoral studies were pursued in a joint supervision between Sao Paulo State University and the University of Paris XI (2008–2012), where he investigated how nanoparticles increased the thermal stability of polymeric materials. After that, he spent three years as a postdoc fellow at the Grunwaldt Group at the Karlsruhe Institute of Technology (2012–2015); there, he investigated the structure-function relationship of nanomaterials applied to catalysis. Currently, he studies the application of nanomaterials & new fertilizers in agriculture, and develops methods to detect and quantify elements in plants & soils systems. He is interested in collaborative research involving X-ray spectroscopy, chemistry, and agriculture.