Quantitative EDX Analysis in TEM. Practical Development, Limitations and Standards

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Abstract: Principles and methods of EDX microanalysis in transmission electron microscopy are presented. Absorption and fluorescence corrections are not carried out in TEM, as real thickness of the sample in the precise analysis point is unknown, but very small. Therefore it is assumed that the effect of such corrections should be minimum. Small thickness guarantees a lack of spread of electrons to larger areas than the spot size used, which means the possibility of very high spatial resolution chemical analyses. In relation to electron microprobe or scanning electron microscope, microanalysis in TEM presents the advantage of minimum size of analysis spot, but its drawback is its lower analytical quality. As the excited volume is an unknown figure, microanalysis in TEM always produces relative results, that is, only the ratios of concentrations among the various elements can be measured but not their absolute concentrations. This is an intrinsic limitation of the method.

Introduction.

Historically, X-ray analysis with an electron beam began with the study of thick samples in which the electron beam is completely absorbed. In contrast, with thin samples the beam passes right through the sample.

Hilier and Baker were the first to suggest that the X-ray signal generated by a focused electron beam could be used to obtain the element composition of a sample [1].

Castain described the equipment ("Electron probe micro-analyzer" = EPMA) as well as the steps for obtaining a quantitative microanalysis of thick samples [2]. Castain's procedure assumes that the concentration of an element (Ci) in a sample generates a certain, characteristic, X-ray intensity. He suggested that, if a standard of known composition (Cst) is taken for element I, we can measure the intensity ratio Ii/Ist, where Ii is the intensity measured in the sample and Ist is the intensity measured in the standard:

$$C_i / C_{st} = K\, I_i / I_{st} \qquad (1)$$

K is a correction factor that includes three different effects: atomic number (Z), X-ray absorption by the sample (A), and X-ray fluorescence by the sample (F). The complete correction procedure is termed ZAF Correction and it is complex, requiring a computer for calculation.

If the analysis is performed on a thin sample that is transparent to the electrons, then the correction procedure is considerably simplified. Factors A and F can be minimized and consequently ignored, and the only factor remaining to be corrected is the atomic number. In addition, if the sample is thin, the volume analyzed is reduced, so that the spatial resolution is much greater (much smaller areas can be analyzed, see below).

Although EDX detectors and transmission electron microscopes (TEMs) equipped with the STEM (scanning transmission electron microscopy) system were developed in 1960, it was not until 1975 that Cliff and Lorimer [3] demonstrated the possibility of doing the afore-mentioned simplification of Castain's original equation (1). In this case it is not necessary to incorporate the intensity data of a standard, as it is enough to obtain the intensity ratio of two elements measured simultaneously by EDX. This step revolutionized the microanalysis of thin samples.

In summary, the difference between EDX microanalyses with TEM and with SEM or EPMA lies in the sample thickness: in SEM and EPMA, the thickness is finite and the ZAF correction (atomic number, absorption and fluorescence) therefore has to be done, whereas with TEM only the Z correction (atomic number) need be carried out.

The ZAF correction is neither necessary nor possible, which is both the simplification and the limitation of TEM microanalysis (since it is difficult to determine the sample thickness at a particular point). Therefore, the analytical quality is not equal to that obtained with SEM or EMPA. In fact, the margins of error for TEM are around 10% to 15%, depending on the sample. The lower detection limit also varies in accordance with the element weight. With elements that are not excessively light, this limit is about 0.3% wt., making it less than ideal to analyze trace elements.

In the case of TEM microanalysis, since the sample is thin (thin sample = 500-1000 Å), the analyzed area is the same as the excited area, that is, equal to the spot size. It is therefore possible to perform microanalyses as small as the electron beam size. The spatial resolution can be a few tens of Å, while in SEM or EPMA it is 1 or 2 ìm. This technique is termed AEM (analytical electron microscopy).

To optimize the spatial resolution at those values, small probe diameters and thin samples must be used. This implies that the counting rate is going to be low, which could be a limiting factor. Normally, an experimental compromise is reached, although there are alternative solutions such as increasing the filament emission.

In accordance with the above factors, there are clearly delimited, although complementary, fields of use for these three techniques (SEM, TEM and EPMA).

Method of Cliff and Lorimer.

The best quantitative analysis approximation for thin samples in a TEM/EDX system has been given by Cliff and Lorimer [3]. This aproximation is based on the assumption that, in thin samples, there is no absorption or fluorescence effect, only that of the atomic number (thin foil criterion). Therefore, these authors relate the ratio of the X-ray intensities of two elements, A and B, to the ratio of concentrations via the following linear equation (the intensity is proportional to the concentration):

$$C_A = K_A I_A \quad (2)$$

$$C_B = K_B I_B \quad (3)$$

dividing

$$C_A / C_B = K_{AB} (I_A / I_B) \quad (4)$$

where

I_A = X-ray intensity of the characteristic line of element A
I_B = X-ray intensity of the characteristic line of element B
C_A = [A] by weight percent of element A
C_B = [B] by weight percent of element B
K_{AB} = K factors of A with respect to B.

The K_{AB} factors depend on the atomic number (Z) of elements A and B and on the accelerating voltage of the microscope. Not all elements produce X-rays with the same ease. If K is high, there are few X-rays, whereas if K is low, the X-rays are higher. A proportionality factor therefore has to be taken into account. The curve relating Z with KAB is characteristic for every electron microscope, every accelerating voltage and every detector.

These factors can be calculated theoretically (the commercial programs for the equipment include theoretical factors) or experimentally (with standards). The calculation is simple, not requiring a

computer, and the background subtraction process is much simpler than for SEM or EPMA since there is less background in thin samples.

The K factors are not radically different than 1 (except for light elements), which allows a qualitative determination of minerals based on the peak height since it gives a simple, direct spectrum interpretation.

Since most of the minerals analyzed in geology are silicates, the factors are usually expressed in relation to Si. Non-silicate compounds can still be analyzed, of course, but it is slighly more complex.

$$\frac{C_A / C_{Si}}{C_B / C_{Si}} = \frac{K_{ASi}}{K_{BSi}} \times \frac{I_A / I_{Si}}{I_B / I_{Si}} \quad (5)$$

Therefore,

$$K_{AB} = K_{ASi} / K_{BSi} \quad (6)$$

This procedure allows us to determine the relative proportions of two elements other than Si.

The results are correctly expressed by the formulas. In EPMA the results are usually given as a proportion of oxides due to the importance of the sums. In TEM that is not possible as the analytical results are relative proportions and the datum of the total is impossible to determine.

C_A/C_B can be determined, but not C_A and C_B. This basic limitation of the method is not a severe problem in mineralogy or similar fields, since the calculation of formulas is always done with normalizations to a known factor such as number of oxygens, charges, total cations, etc. Therefore, the control in TEM analyses is not for the totals, but for the correct formula.

To summarize, microanalysis by EDX with TEM is a relative method in which the results are the atomic proportions between the elements present. To obtain the absolute proportion, we must have information allowing us to obtain a conversion factor, for example, the proportions of a given element, the total number of cations, the total number of positive or negative charges, etc. This is possible to do in minerals that have a formula with some known characteristics.

The precision of the method depends on the calibration of the K factors used. Due to the difficulty in obtaining good standards (see below), the K factors of all the elements are not normally calibrated. Instead, a compromise is reached, determining the elements of greatest interest and interpolating or extrapolating, in the K factor versus Z curve. This method will not work with light elements, particularly elements with an atomic number lower than that of Ca, although it will work with elements with an atomic number higher than that of Ti.

The K factors must be recalibrated when the detector crystal is changed or when the Be window is replaced, since the factors depend on the characteristics of the detector and vary from one to another. However, it is not necessary to recalibrate when a filament or a high-tension tank is changed. It should also be routinely recalibrated every two or three years.

Ways to express the k_{ab} factors as a function of the units of concentration. Definition of factors.

A concentration can be expressed in different ways: As the weight percentage of the element (C_A), as the weight percentage of the oxide (C'_A) or as the atomic concentration of the element (number of atoms) (N_A).

To transform from one type of concentration to another, we use the molecular weight of the oxide and the number of atoms in the oxide formula, or the atomic weight of the element. Traditionally, the calculation in EPMA is performed based on the C'_A (proportion of oxides). To do so, one normally transforms it to N_A, which is a direct datum from TEM or SEM (this datum also appears in many EPMA results).

The equation of Cliff and Lorimer depends on the concentration expression used, resulting in 6 possible definitions of factors [4].

We must know which of these expressions is used by the computer microanalysis program being employed (for instance, EDAX uses C_A, that is, proportions of elements by weight). Regardless of the type of factor used, the program presents the results both as the atomic proportion of the element and as proportions of the element by weight.

The result in atomic proportions is a relative formula, for which we have only to multiply or divide all the concentrations by one factor. The factor is calculated based on the number of oxygens, the number of positive charges, the number of cations, the concentration of a given cation, etc., depending on the mineral to be calculated.

EDAX, for instance, has two types of presentations: The proportion of elements adding up to 100 (expressed in atomic proportions and in element weight) and the proportion of elements plus the oxygen corresponding by stoichiometry adding up to 100, expressed in atomic proportions and in element weight.

Technical aspects and operational conditions for the analysis.

The operational conditions for the analyses should be optimized for each equipment. In general, the following aspects should be taken into account before performing the analyses themselves:

a. Operational method: TEM or nanoprobe for qualitative analyses and STEM for qualitative and quantitative analyses.

b. Aperture of condenser 2: The aperture must be small, top-hat shaped and made of Pt. This shape minimizes the background X-rays from the illumination system. The small size serves to increase the spatial resolution.

c. Goniometric sample holder – analytical: it must be double-tilted and have a low background, with a protective Be capsule. It is crucial to use this type of sample-holder in order to minimize the effects of background radiation, which produces background and exotic peaks in the spectra.

d. Sample tilt: The sample must be tilted so that the X-rays reach the detector, normally a slightly steeper angle than that of the detector so that it can receive the radiation.

e. Selection of a zone for analysis: It is extremely important that the area be thin enough that there are no phenomena of absorption or fluorescence. The thickness is controlled in three ways: seeing a clear diffraction (which guarantees thinness), a count number no higher than a fixed value that depends on the size of the window, the filament emission, type of material, etc., and visually.

Avoid superpositioning of particles to prevent mixed analyses (observe the diffractions and make sure each one corresponds to only one crystal).

It is also important not to be too near the Cu grid divisions or Cu ring to avoid too much Cu in the spectrum, which would decrease the counts from the rest of the elements.

f. Live time: It varies according to the element to be measured: short times for light elements to prevent volatilization problems (15 to 30 sec) and longer times (100 to 200 sec) for other elements.

g. Dead time: It should be under 15%.

h. Background subtraction: This is done manually. It is curved and certain points are chosen corresponding to energies with no important peaks.

i. Presence of Cu and Ar in the spectra: The Cu derives from the ring or grid. The presence of this element should be minimized as much as possible since they subtract from the other elements in the mineral to be analyzed, cause a background in the diagram, and the L line of Cu nearly overlaps that of Na. If the line is very large, then small amounts of Na may go unnoticed.

If the sample is prepared on a grid, the position of the grain in the grid is important as more Cu will appear if it is near the divisions. In contrast, if the sample is prepared by the ion milling method, Ar usually appears in the spectrum. However, it is not normally a drawback, as no interesting peaks fall in the same area.

j. Increase in the filament emission: On certain occasions, when the minerals are very small or provide very few counts, the filament emission can be increased, thus increasing the number of counts.

k. Sample type: The samples used for microanalysis can be prepared with a ring, grid, or as ultramicrotome sections.

Differences between TEM and STEM modes.

Depending on the laboratory, EDX quantitative analyses in transmission microscopes can be carried out in TEM or in STEM mode. Qualitative analyses can be carried out in either mode regardless of the mode chosen for the quantitative analyses. Both methods have advantages and drawbacks.

a. STEM mode: This mode employs a tightly focused beam that scans the chosen sample area, although the beam can also be stationary.

b. TEM mode: This mode consists in choosing an area of the sample, widening or narrowing the beam, and analyzing. The resolution is several tens of nm. Beam damage can be minimized by widening the beam, although then the area analyzed is obviously larger.

STEM mode produces an electronic image where a detector measures the transmited intensity at each point of the sample. The beam is tight-focused and scans a certain area of the sample, constructing an electronic image in a similar way to the SEM mode. The image can be bright field (from the transmited beam) or a dark field (from diffracted beam). The interest for microanalysis is that the performance conditions can be very accurately standardized.

Operational method: A window that will be the area analyzed and a beam size are defined, thus establishing standard working conditions in which the analyses of the standards and of the sample are obtained. The thickness is standardized by the number of counts, although it can also be done visually with a bit of practice.

The advantages of the STEM mode are:

- Precise standardization of the working conditions.
- Absorption and fluorescence are minimized by controlling the sample thickness with the count number.
- Volatilization is minimized.
- Substances that rapidly deteriorate with TEM can be analyzed for longer periods with STEM.

The drawbacks of the STEM mode (or advantages of the TEM mode) are:

- The area is not selected directly on the transmission image, but on an electronic image (there are sometimes difficulties in passing from one to another).
- The scan does not guarantee an equivalent beam time at all points of the window due to the scanning mode, which first scans a line horizontally and then drops to the next lowest horizontal line and so on; thus, the beam does not travel through all the window points the same number of times.
- Changing from TEM mode to STEM mode can be complicated or not depending on the microscope.

Standards.

To determine the constants that relate intensity and concentration, we must use a series of standard

substances for which the composition is perfectly well known and that also fulfill the following characteristics:

- They must be compositionally homogeneous at TEM scale.
- The composition must be known and certified by other methods such as EPMA.
- They must be stable in a vacuum.
- Standard types: they can be natural minerals or chemical compounds.
- Method of preparation: ring or grid depending on the standard.

It is difficult to find good standards as they must be homogeneous at TEM scale, that is, several orders of magnitude below the EPMA scale. There is some difficulty in the preparation method since, in many cases, there is very little standard available.

In standards of light elements, a small thickness must be ensured, since otherwise there is excessive absorption (particularly true with Na). Except with laminar minerals (where the thickness is easily controlled because they exfoliate easily), it is best to prepare samples with ion milling method to find thin zones on the borders of the wedge and thus better control the SAED (selected area electron diffraction) thickness.

The errors in the calculated factors are expressed as percentages (2 x standard deviation)/average value (theoretical error of method = 5% for elements with Z greater than 12 and 30% for Na and K):

$$\text{error (\%)} = [(2 \times \text{standard deviation})\text{Average}] \times 100 \quad (7)$$

As an example, the standards used in the Philips Cm20 TEM-STEM electron microscope of the Centro de Instrumentación Científica of the University of Granada are: Albite (Na, Al), biotite (K, Fe, and Mg), spessartine garnet (Al and Mn), muscovite (Al and K), olivine (Mg and Fe), titanite (Ti and Ca), Mn sulfate (S) and Ca sulfate (Ca).

The factors determined with the same equipment expressed with respect to Si together with the errors (expressed as above) are:

- Large window: Na = 1.28 (5%), Mg = 1.05 (6%), Al = 1.00 (3%), Si = 1, K = 1.17 (4%) (at 15 sec or if it is major) and 1.23 (6%) (at 100 segundos or if it is minor), Ca = 1.16 (3%), Ti = 1.34 (4%), Mn = 1.31 (3%), Fe = 1.24 (4%).
- Small window: Na = 2.04 (4%), Mg = 1.07 (5%), Al = 1.00 (5%), Si = 1, K = 1.22 (5%) (for 15 sec) and 1.36 (9%), Ca = 1.15 (3%), Ti = 1.35 (3%), Mn = 1.29 (3%), Fe = 1.35 (3%).

Method.

1. Identification of peaks: This is performed with tables or with software (the programs have identification routines).

2. Background extraction: This is done manually, establishing specific points through which the background curve must pass and correcting possible errors manually. The zone with Na, Mg, Al and Si is especially difficult since the peaks interfere with each other, the background is larger and intermediate points cannot be established. Therefore, a point is placed at the beginning and end of each of the 4 element area and manually correction is made for possible errors.

3. Measurement of peak intensity: Peak or channel method. This is based on the fact that the peak area is proportional to the intensity of the X-rays.

4. Quantification: The peak area is measured (as the number of counts), the background is subtracted, the proportionality factors are applied, and the results are given as weight and atomic proportions for each element.

Volatilizations.

Irradiation by the electron beam can produce a loss or redistribution of elements in the sample, causing certain elements to become volatile. It primarily affects light or poorly bonded elements (especially Na and

K), although it can also affect heavy elements (i.e. Hg).

The same phenomenon occurs in SEM, EPMA and AEM, although in the latter it is especially significant since the entire element can be lost. Moreover, in AEM it affect more elements and in greater proportions.

The main causes include: a high accelerating voltage, small analysis zones, and defective character of crystals usually studied by TEM.

The following procedures can help to minimize the problem, although nothing can prevent it completely:

1. STEM mode (scanning instead of a fixed beam).
2. Use the largest possible scanning area (if it is small, use a rectangular scan so that the entire grain is covered) and take advantage of possible elongation of the grain.
3. Use short times (15-30 sec) for volatile elements (two spectrum recording)
4. Cool the sample to lessen volatilization.

Volatilization has a greater effect on major elements. For trace elements, use the datum from the longer scanning period, since the peak is very poorly defined in the spectrum in the shorter period.

The presence of volatilization should be assumed and take it into account in the interpretation of the data. Verify whether or not volatilization is actually present with the two spectrum recording (short and long times) and evaluate the effect this could produce on the final data.

Quantitative methods with fluorescence and absorption corrections.

These more sophisticated methods are intended to overcome the main limitation in AEM for greater analytical quality: the effects of absorption and fluorescence. The main problem lies in determining the exact thickness of the sample.

The thickness (t) of the sample is defined as the average thickness parallel to the light axis of the microscope at zero tilt (if the sample is tilted at an angle á, the effective thickness will be equal to t/cosá).

There are many techniques for determining sample thickness:

1. Methods based on diffraction techniques: These can only be applied to single-crystal samples with a very exact sample orientation. Methods based on CBED (thickness fringes counts: bands Kossel-Möllenstedt) [5]. Drawback: It is rare to obtain an accuracy of over 10% and its application is complex.

2. Methods based on the calibration of X-ray intensity in samples of known thickness [6]: The main problem with these methods is the difficulty in reproducing experimental conditions and that a Faraday cup is needed in order to accurately measure the beam current.

3. Methods based on parallel measurements or contamination-spot separation method [7,8]: Contamination in thin samples appears as conical carbonaceous deposits up to 1 ìm high that can be used as markers for the sample surface. The separations between deposits (which appear on both faces of the sample) can be easily distinguished when the sample is steeply tilted. If this angle is known, the local thickness can be deduced. This method will only give the upper thickness limits of the sample and is not very accurate.

4. Method based on continuous X-ray intensity [9]: This is based on the fact that the thickness can be calculated by considering the relations between continuous X-ray intensities in a region of the spectrum without characteristic peaks (10-16 KeV). It is mostly used with biological samples.

5. The most recent attempts at estimating thickness are primarily those of Van Cappellen [10,11]: K factors for distinct unknown thicknesses were determined and attempted to extrapolate them to thickness zero. The different thicknesses are represented by the absolute number of counts. It represents the factors as a function of the thickness and extrapolates to zero. Van Cappellen and Doukham [11] tried to determine the thickness as a function of the relative evolution of the different elements for distinct thicknesses. Analyses are taken at different points for different, unknown thicknesses. The proportions of the elements vary with the thickness. The method establishes curves that are theoretically parabolic and

reconverts the data to positive and negative charges. Since the minerals must be neutral, the point where the curves intersect is zero thickness.

a. Absorption correction:

If there is absorption of X-rays by the sample, then the X-ray intensity detected will obviously be lower than the intensity generated and therefore the concentration of the element is not simply proportional to its intensity. Therefore, the K factors can be modified to take this reduction into account.

If K_{AB} is defined as the true factor when the sample has zero thickness, we can define another factor, K_{AB}^*, when absorption phenomena occur, as follows: $K_{AB}^* = K_{AB}$ (ACF). ACF is the Absorption Correction Factor.

The absorption correction factor has a complex formula that depends on: the coefficient of the mass absorption of the sample elements, the take-off angle, the sample density and the sample thickness. The ACF value is 1 when there are no absorption phenomena and it is over 10% when the absorption phenomena are significant.

The problem with applying it lies in determining the sample thickness (see different methods above). It is possible to use techniques such as that of Van Cappellen [10] and Van Cappellen and Doukham [11] so that the thickness is not a problem (using a simplified absorption factor that does not include the thickness, since it is extrapolated to zero).

b. Fluorescence correction:

Corrections for fluorescence are lesser than those for absorption and, therefore, they are less important for optimizing the analyses. The equation used for the Fluorescence Correction Factor (FCF) was developed by Nockolds et al. [12]. $K_{AB}^* = K_{AB}$ (FCF). Where FCF is the Fluorescence Correction Factor.

References

[1] J. Hilier and R.F. Baker: J. Appl. Phys. Vol. 15 (1944), p. 663.
[2] R. Castain: *Ph.D. Thesis University of Paris* (Onera Publication # 55, France 1951).
[3] G. Cliff and G.W. Lorimer: J. Microsc. Vol. 103 (1975), p. 203.
[4] M. Mellini and R. Menichini: Rend. Soc. Ital. Miner. Petrol. Vol. 49 (1985), p. 261.
[5] P. M. Kelly, A. Jostsons, R.G. Blake and J.G. Napier: Phys. Stat. Sol. Vol. 31 (1975), p. 771.
[6] G. Love, M.G.C. Cox and V.D. Scott: in *Developments in Electron Microscopy and Analysis* (D. L. Misell Ed., Inst. Physics, UK 1977), p. 347.
[7] P.B. Hirsch, A. Howie, R.B. Nicholson, D.W. Pashley and M.J. Whelan*: Electron Microscopy of Thin Crystals* (Butterworths, UK 1965).
[8] G. W. Lorimer, G. Cliff and J. N. Clark: in *Developments in Electron Microscopy and Análisis 1975* (J. A. Venables Ed., Academic Press, UK and EEUU 1976), p. 153.
[9] T.A. Hall and P.R. Werba: in *Electron Microscopy and Analysis* (W. C. Nixon Ed., Inst. Physics, UK 1971), p. 146.
[10] E. Van Cappellen: Microsc. Microanal. Microstruct. Vol 1 (1990), p. 1.
[11] E. Van Cappellen and J. C. Doukham: Ultramicroscopy Vol. 53 (1994), p. 343.
[12] C. Nockolds, M.J. Nasir, G. Cliff and G.W. Lorimer: in *Electron Microscopy and Analysi*s (T. Mulvey Ed., The Institute of Physics, UK 1980), p. 417.