

TEM-EDS microanalysis: Comparison among the standardless, Cliff & Lorimer and absorption correction quantification methods

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ABSTRACT

Available quantification methods for energy dispersive X-ray microanalysis in transmission electron microscopy, such as the standardless method (SLM), the Cliff-Lorimer approximation (CLA) and the absorption correction method (ACM), are compared. As expected, the CLA and ACM give superior results with respect to the SLM. As far as absorption can be considered negligible, CLA and ACM perform similarly. However, starting from mass-thickness of the order of $22 \times 10^{-6} \text{ g/cm}^2$, absorption become significant and the ACM gives better results. More accurate analyses can be obtained with the ACM if distinct $k_{O/Si}$ factors are determined for light and heavy minerals, respectively, placing a divide at 2.90 g/cm^3 . Caution must be used when k -factors are derived indirectly from minerals with very different structure/chemistry, suggesting that separate k -factors data sets are required for accurate EDS quantification, at least for the major and diverse broad classes of minerals. Element diffusion of monovalent cations and channelling effects may represent a complication, especially in very anisotropic minerals such as phyllosilicates, where these two phenomena may occur together.

1. Introduction

Transmission electron microscopy (TEM) is a well-established technique for the study of minerals from the microscale down to the atomic scale. Other than structural and microstructural information, chemical compositions at a comparable scale can be obtained [1–3]. Basically, two different methods exist to extract chemical information from a TEM sample, energy dispersive X-ray spectroscopy (EDS) and electron energy loss spectroscopy (EELS). Although the latter performs better for light elements and possesses a lower detection limit, EDS is the most widespread analytical method installed in TEMs because it is easier to use, faster and of lower acquisition cost. Every element can be reliably identified by EDS with the exception of a few pathological overlaps and light elements such as H, He, and Li. Following elemental identification, the amount of each constituent element can be determined and expressed as a mass or atom per cent.

In electron microprobe analysis of bulk samples, either with a EDS or a WDS (wave dispersive X-ray spectroscopy) system, the raw chemical analysis requires some corrections, generally referred to as ZAF correction [4], where the acronym encapsulates a correction procedure for the effects of atomic number (Z), absorption (A), and secondary

fluorescence (F) that are calculated separately by appropriate physical models. In TEM-EDS microanalysis, the A and F corrections are generally omitted because the sample is so thin (tens of nm), and therefore the interaction volume so small, that they are negligible. This is an assumption called the *thin foil criterion* [5]. However, as it will be demonstrated, this is not always the case, especially for thick and dense samples.

Whereas WDS microprobe analysis of bulk samples requires calibration with standards of known composition [6], EDS analysis can also work without such calibration, making use of the *standardless method*, where the X-ray emission of the targeted element is calculated from *first principles* or *remote standard* method [7–10]. In this case, the raw analytical total must be normalised to unity to compensate for the lack of an electron dose correction, which is assumed by many users to be a source of comfort. The latter, along with the simplicity of operation with "one-button" analysis and the apparent close relation to the conventional standards procedure, are at the basis of the popularity of standardless analysis.

The standardless method can be used either for bulk samples in a scanning electron microscope (SEM) or for thin foils and nanoparticles in a TEM. Although in both cases it reveals very sensitive to the choice of

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the calculation parameters and the analyses can be affected by large errors, whenever the investigated material has a simple and well constrained composition, as in most synthetic materials, or a qualitative analysis is just what is required, the standardless method may be preferred, i.e. the time spent for standardisation is not justified.

However, minerals are commonly more complex than synthetic materials such as semiconductors or metallurgical samples, many elements may be present at the same time, the structure symmetry is lower on average (i.e., the X-ray emission and absorption within the specimen may be orientation dependant), and different minerals may have very different structures (i.e., the matrix effects may be significantly different from mineral to mineral). For all these reasons, in Earth Sciences, EDS analyses obtained through comparison with standards of known composition are preferred.

In order to make valid the thin foil criterion, the analyst is usually forced to work in very thin areas of the sample, with the drawbacks of a low count rate and high electron dose per atom, causing quite high statistical error and elemental diffusion (up to sample amorphization), respectively. To mitigate these problems and whenever one has to deal with thick and/or dense samples, TEM-EDS analyses can be obtained from relatively thick areas, providing a correction for absorption.

In this paper, we test methods commonly used in TEM-EDS analysis quantification on several reference samples, namely the standardless method [11], the Cliff-Lorimer approximation [12] and the absorption correction method based on electroneutrality [13]. Moreover, a spreadsheet based on a parameterless absorption correction approach to EDS spectra of ionic compounds is also presented. The spreadsheet was implemented for use within EXCEL environment and was developed to support mineralogists in their routine task of recalculating the composition of minerals through TEM-EDS.

2. Theoretical background

2.1. The Cliff-Lorimer approximation

The rationale for the quantification of X-ray analysis in analytical transmission electron microscopy (AEM) dates back to the monumental work of Castaing [6], who described a procedure for the quantification of electron-activated X-ray emission from bulk samples that still forms the basis of the quantification routines of today electron microprobe (EMPA). According to Castaing [6], the ratio of the concentration of an element (i) in the unknown (C_i) and in a standard of known composition ($C_{(i)}$) is equal to the ratio of the related measured intensities ($I_i/I_{(i)}$) through a “sensitivity factor” K :

$$\frac{C_i}{C_{(i)}} = [K] \frac{I_i}{I_{(i)}} \quad (1)$$

The sensitivity factor K takes into account the difference between the generated and measured X-ray intensities for both the standard and the unknown, and is affected by the atomic number of the analysed element (Z), the absorption of the X-rays within the specimen (A) and the secondary fluorescence of X-ray within the specimen (F).

The fact that in AEM we deal with electron transparent thin foils instead of bulk specimens has some advantages. The correction procedure can be greatly simplified because, to a first approximation, the A and F corrections can be ignored and only the Z correction is necessary. Moreover, in comparison to the WDS system, the only one available at the time of Castaing [6], where one element is measured at time, the more recent EDS system allows the simultaneous acquisition of the whole spectrum.

Based on these advantages, Cliff and Lorimer [12] developed a quantification method in which there was not the need to incorporate intensity data from a standard, but simply ratio the intensities gathered from two elements (A and B) simultaneously:

$$\frac{C_A}{C_B} = k_{A/B} \frac{I_A}{I_B} \quad (2)$$

In this case, the $k_{A/B}$ factor, often referred to as *Cliff-Lorimer factor*, is related to the atomic number correction factor (Z) only. To obtain the absolute value of C_A , a second equation is required, which for a binary compound is derived simply assuming that $A+B$ constitute the 100% of the specimen:

$$C_A + C_B = 100\% \quad (3)$$

For ternary or higher order systems:

$$C_A + C_B + C_C + \dots = 100\% \quad (4)$$

Of course, k -factors for different elements pairs are related:

$$k_{A/B} = \frac{k_{A/C}}{k_{B/C}} \quad (5)$$

The concentration of the elements could be expressed in atomic% or weight%, or any appropriate units, as long as you are consistent.

2.2. The standardless method

As said, TEM-EDS k -factors may be derived experimentally through standards of known composition, or calculated. The former requires well-characterised, electron-transparent and stable standards, it is slow and more laborious, but normally gives more reliable results (more on this ahead). The latter is quick and painless and represents the basis of the standardless quantification analysis.

The standardless analysis simplifies the measurement process by requiring the analyst to measure only the EDS spectrum of the unknown, whereas all the other operations (i.e. peak integration, background subtraction, etc.) are executed as for conventional analyses with element standards. At least two different methods to standardless analysis are possible: first principles and remote standard [7,8]. In the first case, pure theoretical calculations based on physical parameters are used. The electron-excited X-ray emission from the element A in the specimen is given by Williams and Goldstein [11]:

$$I_A = N \left(\frac{Q\omega a}{W} \right)_A C_A \Delta \rho t \int_0^t \varphi_A(\rho t) e^{-\chi \rho t} (1 + \delta_A) d(\rho t) \quad (6)$$

Where the terms before the integral represent the X-ray emission from the element A in an isolated thin film of thickness Δ and mass thickness ρt ; N is the Avogadro number, Q the ionisation cross section, ω the fluorescence yield for the characteristic X-rays, W the atomic weight and C_A the weight fraction of the element A , a the relative transition probability (the chances that a K_α or a K_β could be produced); the term $\varphi_A(\rho t)$ is the depth distribution of X-ray production and $e^{-\chi \rho t}$ accounts for the X-ray absorption in the specimen (where $\chi = \mu/\rho \cos \alpha$ is the mass-absorption coefficient for a X-ray take-off angle α); the term $(1 + \delta_A)$ accounts for the secondary fluorescence (the X-ray from A may also be fluoresced by other characteristic X-rays of higher energy).

The Cliff-Lorimer approximation assumes that we can measure two characteristic X-ray intensities simultaneously and therefore we can ratio two equations like Eq. (6). Moreover, in thin foils, the F and A corrections are negligible, i.e. the integral terms in Eq. (6) can be assumed equal to unity, obtaining:

$$\frac{I_A}{I_B} = \frac{C_A (Q\omega a)_A W_B}{C_B (Q\omega a)_B W_A} = \frac{C_A}{C_B} \frac{W_B}{W_A} (Z) \quad (7)$$

The above equation can be compared with Eq. (2) and rearranged:

$$k_{A/B} = \frac{1}{Z} = \frac{(Q\omega a)_B W_A}{(Q\omega a)_A W_B} \quad (8)$$

From the above equation one realises that k -factors are mostly

influenced by experimental factors such as: (i) the accelerating voltage, since it affects the ionisation cross section (Q); (ii) the Z number of the measured element, since it influences the fluorescence yield (ω) and the atomic weight (W) and (iii) the relative transition probability (a) of the same series of X-rays.

Eq. (8) needs to be further corrected for the detector efficiency (ϵ_A/ϵ_B), i.e. the absorption the X-rays generated by element A and B undergo passing through the detector components, that we know is higher for light elements (long X-rays wavelength) than for heavy elements (short X-ray wavelength). For all the detector component materials appropriate mass absorption coefficients are required.

On the other hand, the remote standard method for standardless analysis combines the measurement of pure element or stoichiometric binary compound standards with physical models to create a database of intensities. These measurements must be carried out on a particular electron probe EDS system in which the measurement conditions such as the beam energy, the incidence angle, the electron dose, the take off angle and the EDS efficiency are very well known. The intensities so collected need to be corrected for the local EDS efficiency. Then, when the user requires a standard at a different beam energy or that is not present in the library, it is possible to obtain the missing value through a series of mathematical calculations [7,8]. The remote standard method is the most used method for standardless analysis since returns more accurate analysis than the first principle method [9,10].

Of course, for reliable calculated k -factors, accurate values for Q , ω , a and ϵ are required. Amongst these, the most challenging is Q , for which different opinions exist about the way to obtain the best values in the 100–400 kV range relevant for AEM [14,15]. The combination of uncertainties in Q and in the detector parameters is the reason why calculated k -factors are usually not better than ± 10 –20% [5].

2.3. Absorption correction

In dense and/or thick specimens, the thin foil criterion may not be valid. This means that the measured intensity for an element A is less than the generated intensity and the retrieved concentration C_A is no longer simply proportional to I_A . An absorption correction factor (A) is therefore required to take into account the reduced measured intensity. Assuming that the depth distribution of X-ray production ($\varphi_A(\rho t)$) does not change significantly and it is close to unity even for thick samples, from Eq. (6) and following we get [5]:

$$A = \left[\frac{\left(\frac{\mu}{\rho}\right)_A}{\left(\frac{\mu}{\rho}\right)_B} \right] \left\{ \frac{1 - e^{-\left(\frac{\mu}{\rho}\right)_B \rho t \csc \alpha}}{1 - e^{-\left(\frac{\mu}{\rho}\right)_A \rho t \csc \alpha}} \right\} \quad (9)$$

Therefore, in order to calculate correct k -factors, also the mass-absorption coefficients (μ) in the specimen for every measured element X-ray are required, as well as the sample density ρ and thickness t .

The major variable in the absorption correction is the path length $t' = t \csc \alpha$, where α is the take-off angle (Fig. 1). There are several methods to determine the sample thickness at the measuring point, summarised in Williams and Carter [5], but none of them is of practical use when dealing with the quantification of a large number of analyses. Other methods have been developed that avoid the problem of measuring the thickness, known as extrapolation techniques [16–18], but with these methods one must know the beam current, which may be not as straightforward as it may sound. It is worth to mention also the existence of the so called ζ -factor method, which presents several advantages over the Cliff-Lorimer and other methods, such as the possibility of the in-situ thickness determination from the built-in absorption correction if a pure element thin film standard is used [19,20]. However, despite the advantages, that are well described by Watanabe and Williams [20], the main limitation of the ζ -factor method remains the necessity of the beam current measurement.

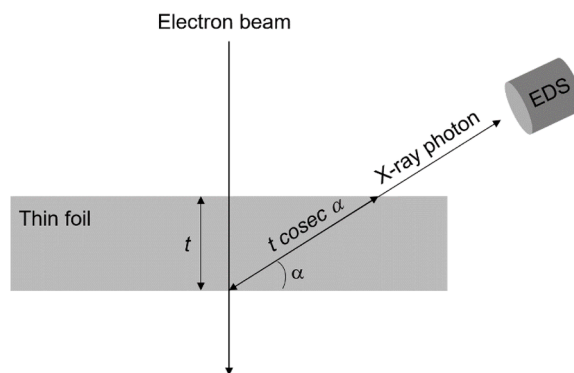


Fig. 1. X-ray photon absorption path within the specimen as function of the thin foil thickness and detector take-off angle α .

To overcome the problem of knowing the thickness and the beam current, an alternative method based on the *electron neutrality criterion* has been developed by Van Cappellen and Doukhan [13], in which no direct measurement of either thickness or beam current is required. Even the density of the sample and the take-off angle are not required, unless one needs to know the real thickness of the analysed area for some other reasons. This method has been adopted in this paper and is here briefly described.

Classical software for X-ray microanalysis usually incorporate a routine based on Eq. (9), i.e. a quadratic approximation for absorption correction. In this equation, μ_A and μ_B are concentration-dependant mass-absorption coefficients. In practice, concentrations are evaluated via successive iterations where μ_A and μ_B updates are used to calculate new correction factors, until the whole procedure converges to what is believed to be the real composition of the target. As matter of fact, if one processes a specific spectrum with varying thicknesses and an approximate density ρ , the computed concentrations will align perfectly on a parabolic curve when plotted versus arbitrary thickness inputs (Fig. 2A). Therefore, with three chosen (fictive) thicknesses, it is possible to mathematically determine the parabola, i.e. predicting how the concentrations vary with thickness.

It should be realised that minerals, as well as ceramics and oxides, are ionic compounds, which means that the sum of all anions and all cations times their respective valence states should be equal. Of course, only one composition on the parabola will meet this condition, in principle only that at the actual thickness, which should be the correct one. Therefore, to determine the actual thickness, it is necessary to calculate the variation of the positive charge and of the negative charge as a function of thickness and observing where the two curves, still parabolic but with opposite slope, intersect (Fig. 2B). However, if the employed density is not the actual density, the retrieved thickness is not the actual thickness, but the composition is still correct (a spreadsheet to evaluate the thickness corresponding to sample electron neutrality is provided as supplementary material SM1).

It should be noted that quadratic functions generally employed in TEM-EDS software are based on first order approximations of the intensity attenuation factor and give a good evaluation of the specimen thickness in a range occurring well before the parabolic maxima or minima are reached. Consequently, data processing should not be attempted if thickness greatly surpasses half the extremity values for the parabolas. In these cases, EDS systems should be equipped with absorption correction equations incorporating higher-order terms.

3. Instrument and samples

The study was conducted at the Platform of Microscopy of the University of Milano-Bicocca using a JEOL JEM 2100P with conventional LaB₆ source, operated at 200 kV. The instrument is equipped with a Gatan Rio9 CMOS camera and an Oxford SSD UltimMax EDS detector

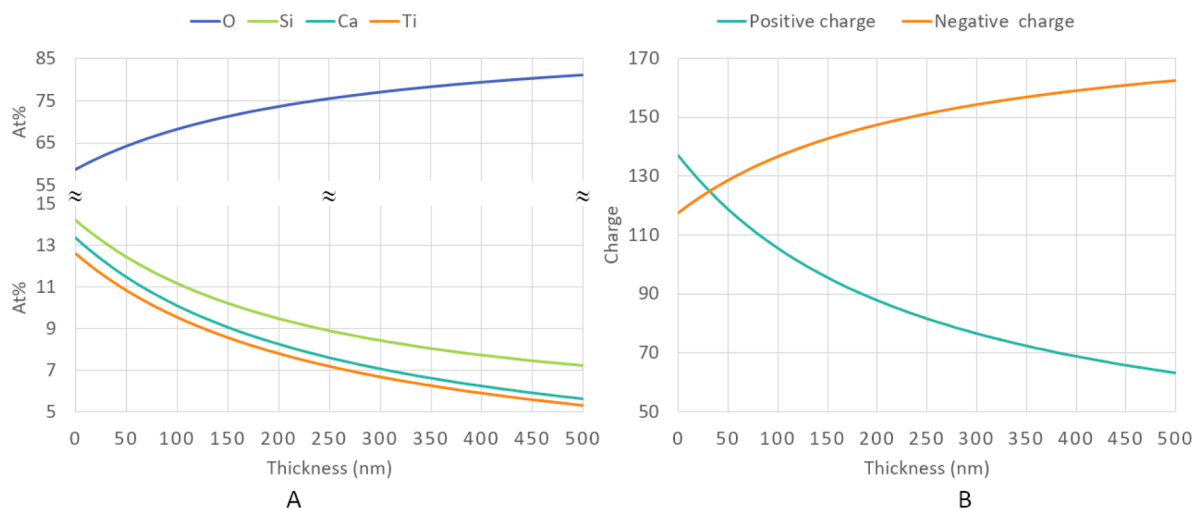


Fig. 2. Quantification of a titanite (CaTiSiO₅) spectrum with the Aztec Oxford software as function of thickness for an assumed density $\rho = 3.55 \text{ g/cm}^3$: (A) Atoms per cent and (B) positive and negative charges. The point where the two curves cross represents the actual foil thickness (30 nm).

with an 80 nm² sensor and windowless design. Both ion-milled TEM-mounts and powdered samples dispersed on holey-Cu grids were employed. The formers were attained by a Gatan PIPS II Cool instrument operated at 5 kV and 10° incidence during the attack step, then progressively lowered during the finalizing step. Ion milled samples were carbon coated with a 10 nm C film to avoid electrostatic charging during the measurements.

The analyses were acquired in STEM mode (scanning–transmission) under the same operating conditions for all standards and test samples: same condenser lens diaphragm (150 μm); magnification (120 kX); camera length (120 cm) and spot size (3 nm). The typical probe current density was in the order of 8.5 pA/cm² (estimated through the fluorescent screen) and the photon count rate in the order of 800–8000 cps (depending on sample composition and thickness). The analyses were taken at 0-tilt orientation with the aim to warrant the same geometry for all standards and test samples, which in almost all cases corresponded to an orientation far from a zone axis, but from phyllosilicates, which were prepared by ion milling from cleavage flakes glued on Cu rings, implying an orientation at 0-tilt close to [001].

k -factors were derived for thirteen elements using fourteen different minerals and two synthetic compounds, all well characterised by EMPA (Table 1). EDS spectra were acquired using a selected area of 50 × 50 nm and an acquisition time of 50 s for all elements. In addition, for Na and K, in order to evaluate any elemental diffusion that commonly affects these elements, an acquisition time of 10 s was also used.

4. Results and discussion

4.1. Experimental determination of k -factors

The experimental determination of k -factors requires, for all the elements of interest, TEM reference standards of known composition [i.e. 21–23] that must fulfil some requirements. The sample must be stable under the electron beam, possibly a single phase, and capable of being thinned to electron transparency. The composition must be homogenous and well characterised and not subject to chemical changes during the milling process or the analysis. Then Eq. (2) is used to retrieve the $k_{A/B}$ values from the measured intensities.

We experimentally derived two different sets of k -factors: (i) one set intended to be used with the Cliff-Lorimer approximation without absorption correction, derived from point analyses taken in thinnest parts of the specimen; and (ii) a second set to be used with the absorption correction method and derived from point analyses taken at various foil thicknesses in the specimen and extrapolated to 0-thickness (Fig. 3). The latter implies that oxygen is analysed and a $k_{O/Si}$ determined. The first set of k -factors and the second set are reported in Tables 2 and 3, respectively. In Fig. 4 we plot the variation of k -factors as function of the analysed element, i.e. as function of the line X-ray energy used for quantification.

The k -factors increase almost regularly on both sides of Si (reference element, $k = 1$), with few exceptions: Cr, which is higher than expected,

Table 1
Minerals and synthetic (*) compounds used as reference standards in the EDS calibration.

#	Mineral/Compound	Composition	TEM mount	Calibrated Element
1	Anorthite	(Ca _{0.95} Na _{0.05}) _{1.00} (Fe _{0.02} Al _{1.96}) _{1.98} Si _{2.03} O ₈	Grid	Al, Ca, O
2	Anorthoclase	(Ca _{0.04} Na _{0.80} K _{0.13} Fe _{0.02}) _{0.99} Al _{1.00} (Al _{0.05} Si _{2.95})O ₈	Grid	Na, Al and O
3	Apatite	Ca _{4.98} P _{2.97} Na _{0.04} Al _{0.01} Si _{0.03} O ₁₂ F	Grid	P
4	Augite	(Ti _{0.02} Na _{0.09} Ca _{0.61} Al _{0.16} Fe _{0.10} ³⁺) _{0.98} (Mg _{0.89} Fe _{0.10} ²⁺) _{0.99} (Al _{0.17} Si _{1.83}) _{2.00} O ₆	Grid	Mg, Al, Ca, Fe, O
5	Biotite	(Na _{0.04} K _{0.90}) _{0.94} (Ti _{0.16} Mg _{1.26} Fe _{1.18} Al _{0.25}) _{2.85} (Al _{1.21} Si _{2.79}) _{4.00} O ₁₀	ion milled	Mg, Al, K, Fe, O
6	Scapolite	K _{0.18} Na _{1.47} Ca _{2.13} Fe _{0.02} Al _{4.32} Si _{7.28} O ₂₄	Grid	Na, Al, Ca, O
7	Spessartine	(Mn _{2.48} Fe _{0.38} ²⁺ Ca _{0.15} Mg _{0.04}) _{3.05} (Ti _{0.02} Fe _{0.21} Al _{1.71}) _{1.94} Si _{3.01} O ₁₂	Grid	Al, Mn, O
8	Hemimorphite	Zn ₄ Si ₂ O ₇ (OH) ₂ H ₂ O	Grid	Zn, O
9	Microcline	(K _{0.90} Na _{0.12}) _{1.02} Al _{1.00} Si _{2.99} O ₈	Grid	Al, K, O
10	Muscovite	(Na _{0.11} K _{0.84}) _{0.95} (Ti _{0.01} Mg _{0.02} Fe _{0.08} Al _{1.92}) _{2.03} (Si _{3.08} Al _{0.92}) _{4.00} O ₁₀	ion milled	Al, K, O
11	Rhodonite	(Fe _{0.21} Mg _{0.01} Ca _{0.09} Mn _{0.72}) _{1.03} Si _{0.98} O ₃	Grid	Mn, Fe, O
12	Titanite	Ca _{1.04} Ti _{0.98} (Al _{0.07} Si _{0.95}) _{1.02} O ₅	ion milled	Ca, Ti
13	Osumilite	(Na _{0.13} K _{0.86}) _{0.99} (Ca _{0.01} Ti _{0.02} Mg _{1.47} Fe _{0.90} Al _{2.67}) _{5.07} (Si _{10.17} Al _{1.83}) _{12.00} O ₃₀	Grid	Mg, Al, K, Fe, O
14	Chromite	(Mg _{0.74} Fe _{0.35}) _{1.09} (Cr _{1.55} Al _{0.38}) _{1.93} O ₄	Grid	Cr
15	CaSO ₄ *	CaSO ₄	Grid	S
16	MnSO ₄ *	MnSO ₄	Grid	S

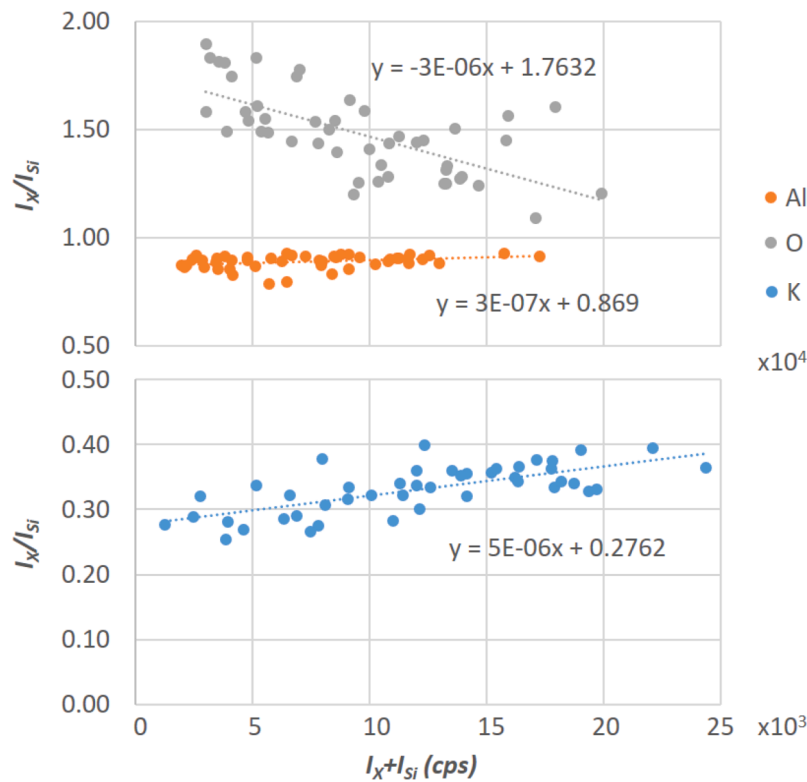


Fig. 3. Example of extrapolation of (I_X/I_{Si}) intensity ratios in muscovite for the determination of k -factors for the elements Al, O and K.

Table 2

k -factors determined using the Cliff-Lorimer approach analysing only the thinnest parts of specimens (in brackets: average of the standard deviations obtained for each mineral-derived k -factor).

	Na	Mg	Al	P	S	K	Ca	Ti	Cr	Mn	Fe	Zn
Anorthite			1.03				1.18					
Anorthoclase	1.16		1.1									
Apatite				1.11								
Augite		1.09	0.99				1.14				1.52	
Biotite		1.06	1.02			1.05*					1.41	
Scapolite	1.19		1.01				1.17					
Spessartine			1.05							1.47		
Hemimorphite												1.56
Microcline			1.08			1.18						
Muscovite			1			1.16						
Rhodonite										1.5	1.44	
Osumilite		1.1	1.05			1.16					1.46	
Chromite									1.58			
CaSO ₄					1.09							
MnSO ₄					1.13							
Titanite							1.18	1.37				
Average	1.18 (0.05)	1.08 (0.04)	1.04 (0.04)	1.11 (0.04)	1.11 (0.03)	1.17 (0.04)	1.17 (0.04)	1.37 (0.04)	1.58 (0.04)	1.49 (0.04)	1.46 (0.05)	1.56 (0.06)

*Outliers not considered in the average.

and K, which shows high variability from mineral to mineral. The $k_{Cr/Si}$ has been determined indirectly through Eq. (10) from the $k_{Cr/Fe}$ of chromite (*chr*), where the $Cr_{K\alpha}$ radiation is strongly absorbed by Fe, and the $k_{Fe/Si}$ in silicates (*sil*), where the $Fe_{K\alpha}$ is not so strongly absorbed, because of the relatively lower average density of silicates:

$$k_{Cr/Si} = k_{Cr/Fe} \cdot k_{Fe/Si} = \left(\frac{C_{Cr}}{C_{Fe}} \frac{I_{Fe}}{I_{Cr}} \right)_{chr} \left(\frac{C_{Fe}}{C_{Si}} \frac{I_{Si}}{I_{Fe}} \right)_{sil} \quad (10)$$

Assuming that the concentrations in the standards are correct, the overestimation of the $k_{Cr/Si}$ factor may be due to the underestimation of I_{Cr} in chromite. This observation uncovers possible uncorrected matrix effects that may affect EDS analysis when dealing with very different

mineral groups.

The high variability of the $k_{k/Si}$ values may be due to the high volatility of K, monovalent and with high ionic radius, under the high energy electron beam. This leads to an overestimation of the factor as it seems the case for muscovite (Table 3) but does not justify the low value determined for biotite (Tables 2 and 3). Other reasons must be addressed for the latter. One of these may be related to the orientation-dependant probability of X-ray generation, sometimes indicated as *channelling effect*, which is pronounced when the analysing beam is parallel or close to a zone axis [24].

The extrapolated 0-thickness values (Table 3) are consistent with the Cliff-Lorimer factors (Fig. 4), recording, in general, slightly higher

Table 3
k-factors determined extrapolating to 0 thickness analyses taken also on thick areas of specimens.

	Na	Mg	Al	P	S	K	Ca	Ti	Cr	Mn	Fe	Zn	O
Anorthite			1.05				1.20						1.27
Anorthoclase	1.22		1.22*										1.24
Apatite				1.12									
Augite		1.08	0.99				1.15				1.54		1.42
Biotite		1.06	1.06			1.06*					1.54		1.17
Scapolite	1.21		1.02				1.13						
Spessartine			1.07							1.51			1.44
Hemimorphite												1.61	1.44
Microcline			1.12			1.20							1.38
Muscovite			1.02			1.38*							1.15
Rhodonite										1.52	1.46		1.52
Osumilite		1.14	1.05			1.15					1.48		1.43
Chromite									1.64				
CaSO ₄					1.08								
MnSO ₄					1.15								
Titanite							1.21	1.39					1.57
Average	1.22	1.09	1.05	1.12	1.12	1.18	1.17	1.39	1.64	1.52	1.51	1.61	1.37

*Outliers not considered in the average.

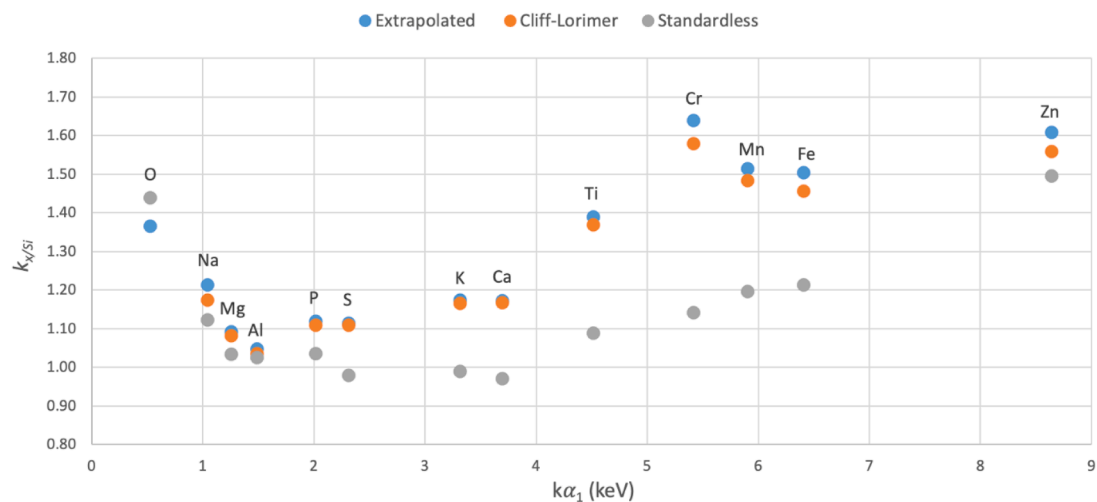


Fig. 4. Plot of the $k_{X/Si}$ factors from spectra acquired in the thinnest part of the sample (Cliff-Lorimer) and from thicker samples extrapolated to 0 thickness, as function of Z.

values, as expected. Major deviations affect heavy elements, Mn, Fe and Zn, since the dense media in which these elements are included may show auto-absorption effects even in thin foils. For Na, which also shows a small deviation, absorption may be significant even in thin foils because of the low energy of the $Na_{K\alpha}$ radiation. Moreover, for this element, diffusion may be significant under the electron beam, in thin foils more than in thick areas, because of the higher electron dose per atom.

Within the same element, stands out the high $k_{Al/Si}$ determined for anorthoclase (1.22), higher than for all the other minerals (0.99–1.12). Due to the closeness in energy between the $Al_{K\alpha}$ and $Si_{K\alpha}$ (1.740 and 1.487 keV, respectively), a value closer to 1 would be expected. A possible explanation of this inconsistency calls into play chemical zonation in the standard, which is almost unavoidable in plagioclase, and that was actually detected, but evidently not side-stepped!

Finally, the $k_{O/Si}$ shows quite scattered values in comparison to those of other elements. This can be easily understood considering the very low energy of the $O_{K\alpha}$ radiation (0.525 keV), which is easily absorbed and to a different extent in mineral structures with significantly different density. Because of this difficulty, two different data sets were derived, to be used in different situations: one based on denser phases (augite, spessartine, osumilite, hemimorphite, rhodonite and titanite), whose $k_{O/Si}$ factors range from 1.42 to 1.57, and one based on lighter phases

(anorthite, anorthoclase, biotite, microcline and muscovite), whose $k_{O/Si}$ values range from (1.15 and 1.38). For some unexplained reasons, osumilite (specific gravity 2.58–2.68) behaves as a denser phase.

4.2. Applications to some reference samples

The average k -factors obtained as described above (last line in Tables 2 and 3) were tested against minerals of known composition, viz. johannsenite, antigorite, biotite, cordierite, fayalite and spinel, in order to compare the standardless quantification method (SLM), the Cliff-Lorimer approximation (CLA) and the absorption correction method (ACM). In Table 4 we report TEM-EDS results for these reference samples along with their bulk chemical composition obtained with EMPA (before crushing or ion-milling). For the determination of the thickness with the ACM [13], densities of 3.52, 3.09, 2.60, 2.52, 4.39 and 3.87 g/cm³ were assumed for johannsenite, biotite, cordierite, antigorite, fayalite and spinel, respectively.

As expected, CLA and the ACM return more accurate results than SLM. For CLA and ACM, deviations from the reference samples are within 7 and 8% for major elements, with few exceptions, and commonly around 4 and 5%; they are several times higher for the STL method. The exceptions concern Al in antigorite, iron in johannsenite and K in biotite. The Al content in antigorite, however, is generally low

Table 4

TEM-EDS analyses (a.p.f.u) quantified with SLM, CLA and ACM for reference samples johannsenite, antigorite, biotite, cordierite, fayalite and spinel (averaged number of analysis in brackets).

	Johannsenite (12) 14–104 nm				Antigorite (18) 32–196 nm									
	Reference [*]	STL	CLA	ACM	Reference [§]	STL	CLA	ACM						
Mg	0.04	0.01	75%	0.01	75%	0.01	75%	2.63	1.72	35%	2.49	5%	2.53	4%
Al	0.02	b.d.l.	–	b.d.l.	–	b.d.l.	–	0.07	0.08	14%	0.12	71%	0.12	71%
Si	1.98	1.86	6%	1.97	1%	1.99	1%	1.97	1.49	24%	2.08	6%	2.06	5%
Ca	1.00	0.80	20%	1.02	2%	1.00	0%	0.01	b.d.l.	–	b.d.l.	–	b.d.l.	–
Mn	0.87	0.62	29%	0.81	7%	0.81	7%	–	–	–	–	–	–	–
Fe	0.10	0.17	70%	0.22	120%	0.22	120%	0.10	0.07	30%	0.12	20%	0.11	10%
	Biotite (18) 8–285 nm				Cordierite (18) 48–164 nm									
	Reference [#]	STL	CLA	ACM	Reference ^{&}	STL	CLA	ACM						
Na	0.05	0.07	40%	0.09	80%	0.06	20%	0.07	0.01	86%	0.01	86%	0.01	86%
Mg	1.25	0.98	22%	1.22	2%	1.30	4%	1.52	1.32	13%	1.56	3%	1.60	5%
Al	1.30	1.03	21%	1.23	5%	1.30	0%	3.91	3.55	9%	4.09	5%	4.11	5%
Si	2.84	2.35	17%	2.82	1%	2.74	4%	4.99	4.27	14%	4.85	3%	4.84	3%
K	0.87	0.50	43%	0.68	22%	0.76	13%	–	–	–	–	–	–	–
Ca	0.01	b.d.l.	–	b.d.l.	–	b.d.l.	–	–	–	–	–	–	–	–
Ti	0.22	0.17	23%	0.25	14%	0.24	9%	–	–	–	–	–	–	–
Mn	0.03	0.02	33%	0.03	0%	0.04	33%	0.01	0.00	–	0.00	–	0.00	–
Fe	1.36	0.96	29%	1.36	0%	1.38	1%	0.59	0.42	29%	0.57	3%	0.56	5%
	Fayalite (17) 61–214 nm				Spinel (20) 34–380 nm									
	Reference [§]	STL	CLA	ACM	Reference [§]	STL	CLA	ACM						
Mg	0.03	0.03	0%	0.03	0%	0.03	0%	0.71	0.57	20%	0.64	10%	0.67	6%
Al	–	–	–	–	–	–	–	1.95	1.77	9%	1.94	1%	1.95	0%
Si	0.99	0.98	1%	0.94	5%	0.95	4%	–	–	–	–	–	–	–
Mn	0.09	0.09	0%	0.10	11%	0.10	11%	0.01	0.01	0%	0.01	0%	0.01	0%
Fe	1.90	1.70	11%	1.97	4%	1.93	2%	0.36	0.34	6%	0.44	22%	0.39	8%
Zn	–	–	–	–	–	–	–	0.01	0.01	0%	0.01	0%	0.01	0%

*[27]; §[28]; # [29]; &[30]; §from the mineralogical collection of the Department of Earth and Environmental Sciences (DISAT) of the University of Milano-Bicocca.

and the deviation may arise from a poor counting statistics affecting both, EMPA and TEM-EDS. Moreover, the crystal analysed with TEM-EDS comes from the same suite of crystals analysed by EMPA, but it was not directly analysed by EMPA, i.e. a deviation in composition is not surprising, especially if one considers that the Mg/Si ratio may be affected by polysomatic disorder. Johannsenite is markedly zoned; the zoning concerns relative abundances of Mn, Fe and Mg in the M1 site [25,26]. Since a large deviation affects also Mg and, to a lesser extent Mn, and considering that, as for antigorite, the crystal analysed by EMPA is not the same analysed by TEM-EDS, the observed TEM-EDS composition may be real. The large deviation affecting the K content in biotite, where it is underestimated, may be due to diffusion under the highly focused electron beam. In principle, if the analysis of the unknown is performed on the same matrix used for the standardization and under the same experimental conditions, diffusion should affect in the same manner both standard and unknown, cancelling each other. In this case, the same experimental conditions were applied, but the $k_{K/Si}$ was derived from feldspar and osumilite, because of the odd data obtained from phyllosilicates, probably affected by channelling (see above). Therefore, the analysis of interlayer cations in phyllosilicates may pose serious quantification problems because of concurring effects such as diffusion and channelling.

The comparison between ACM and CLA results for fayalite (heavy mineral) and cordierite (light mineral) shows that the number of atoms per formula unit (a.p.f.u) on varying the thickness of the analysed volume is constant in the case of ACM results for both minerals (Fig. 5), as it should be. On the contrary, CLA results show different trends for heavy and light minerals. In fayalite, the a.p.f.u. of Fe and Si diverge with increasing thickness, showing a positive slope for Fe and a negative slope for Si, meaning that Fe is overestimated and Si underestimated with increasing thickness because the $Fe_{K\alpha}$ is less adsorbed than the $Si_{K\alpha}$. The divergence is observable at sample thicknesses as low as 50 nm. This behaviour is only slightly noticed in cordierite for Mg and Fe, i.e. the most contrasting element in terms of emission photon energy in the mineral, and only for thickness higher than 80–100 nm, whereas Al and

Si, with close emission photon energy, evolve in parallel. These observations suggest that absorption correction is effective only for heavy minerals and/or at high sample thicknesses. In cordierite, however, a different phenomenon was observed, which is reflected by the trembling trend of a.p.f.u. as function of thickness and which is due to chemical zoning. A possible exchange vector of the type $Si^{4+} + (Mg, Fe)^{2+} = 2Al^{3+}$ is suggested by the perfect anti-correlation relationship involving these elements (Fig. 6).

5. Conclusions

The standardless method, the Cliff-Lorimer approximation and the absorption correction method normally used in TEM-EDS analysis quantification have been tested on several reference samples. Before, experimental k -factors [i.e. 21–23] and 0-thickness extrapolated k -factors [17] have been determined. The CLA and ACM give superior results with respect to the SLM and, as far as the absorption correction can be considered negligible, very similar. However, starting from mass-thickness of the order of $22 \times 10^{-6} \text{ g/cm}^2$ (estimated on fayalite) absorption becomes significant and the ACM gives better results.

The determination of the $k_{O/Si}$ factor is challenging because the low-energy $O_{K\alpha}$ radiation is severally absorbed and to a different extent in different minerals, leading to scattered data. This problem forced us to determine two different $k_{O/Si}$ to be used in different contexts: one with lighter minerals and one with heavier minerals ($\rho > 2.90 \text{ g/cm}^3$).

Caution must be used when k -factors are derived indirectly from minerals with very different structure/chemistry, as in this study has been the case for the $k_{Cr/Si}$ determined indirectly from the $k_{Cr/Fe}$ of chromite and the $k_{Fe/Si}$ from silicates. The retrieved $k_{Cr/Si}$ deviates considerably from the trend depicted by all the other factors, probably because of the strong absorption of the $Cr_{K\alpha}$ by the dense spinel matrix. This observation lets one suppose that for accurate EDS quantification, separate k -factors data sets are required, at least for the major and diverse broad classes of minerals, such as silicates and metal-oxides.

Some monovalent element with large ionic radius, such as Na and K,

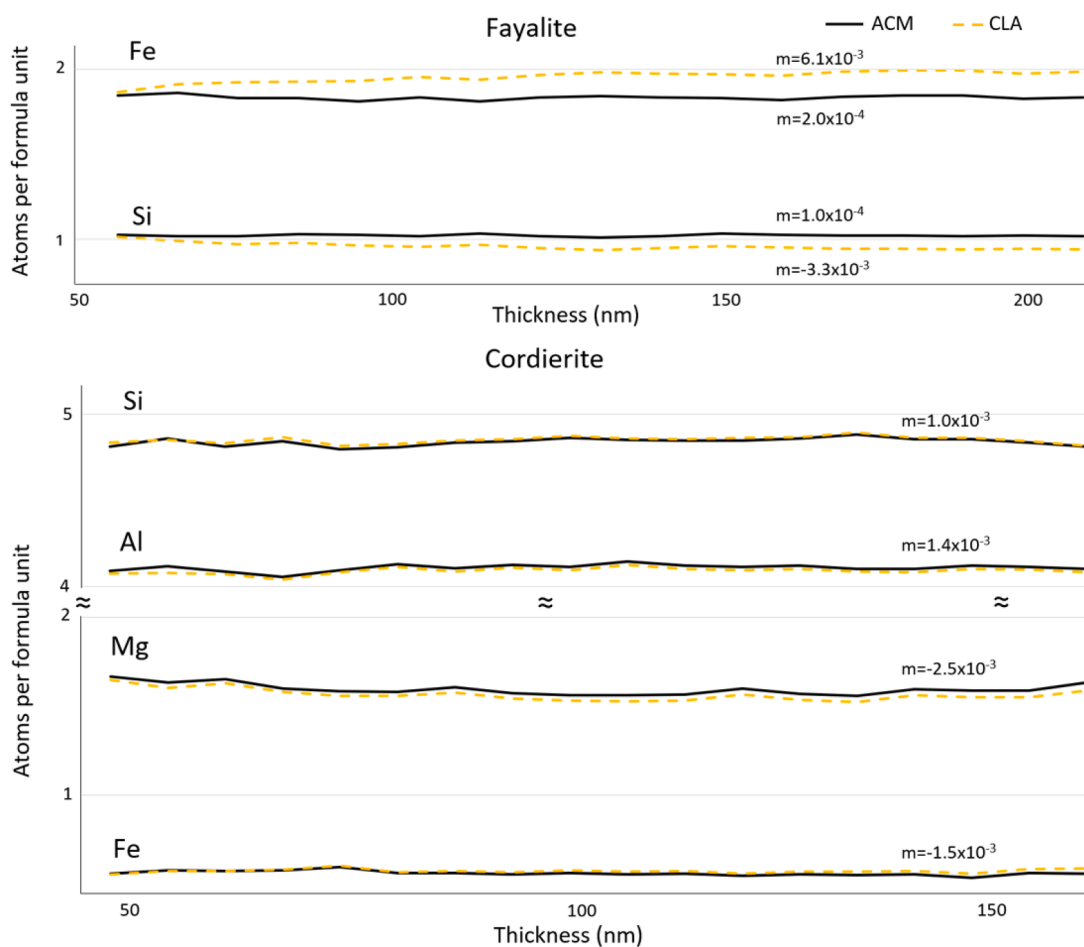


Fig. 5. Comparison of the a.p.f.u. determined with ACM and CLA as function of the thickness of the analysed volume for fayalite and cordierite. In the plots, m represents the slope of the fitted linear trends; for cordierite only one value is given since the ACM and CLA trends almost overlap.

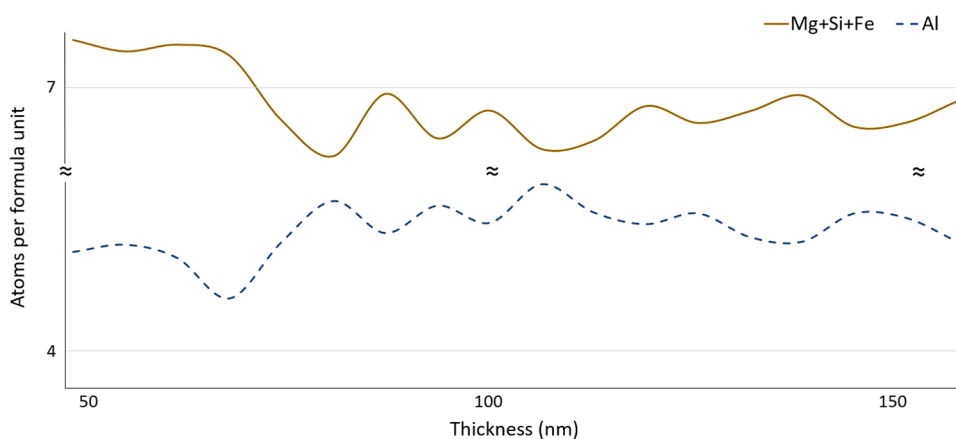


Fig. 6. A.p.f.u. of Al and Mg+Si+Fe measured in cordierite with the ACM as function of the thickness of the analysed volume, showing perfect anti-correlation.

may show diffusion under the highly focused electron beam of the TEM, leading to a decrease with time of the related count rate. For these elements, shorter acquisition times [31] and larger spot analyses, both during standardization and measurement, are required in order to get more reliable data. Finally, for very anisotropic mineral groups, such as phyllosilicates, channelling effects must be taken into account, for instance controlling the orientation of the mineral and reproducing the same conditions during standardization and measurement.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

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