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Microbeam analysis — Electron probe microanalysis — Guidelines for the specification of certified reference materials (CRMs)

Analyse par microfaisceaux — Microanalyse par sonde à électrons — Lignes directrices pour les spécifications des matériaux de référence certifiés (CRM)

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 202, *Microbeam analysis*, Subcommittee SC 2, *Electron probe microanalysis*.

This second edition cancels and replaces the first edition (ISO 14595:2003), which has been technically revised. It also incorporates Technical Correction ISO 14595:2003/Cor 1:2005.

Introduction

For electron probe microanalysis (EPMA), a comparative quantitative analytical method used throughout the world, certified reference materials (CRMs) play a crucial role in the analytical accuracy.

This International Standard has been developed to facilitate international exchange and compatibility of analysis data in EPMA.

It gives guidance on evaluating and selecting reference materials (RMs), on evaluating the extent of heterogeneity and stability of RMs, and it gives recommendations for the determination of the chemical composition of RMs for production as EPMA-certified reference materials.

Microbeam analysis — Electron probe microanalysis — Guidelines for the specification of certified reference materials (CRMs)

1 Scope

This International Standard gives recommendations for single-phase certified reference materials (CRMs) used in electron probe microanalysis (EPMA). It also provides guidance on the use of CRMs for the microanalysis of flat, polished specimens. It does not cover organic or biological materials.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO Guide 31:2000, *Reference materials — Contents of certificates and labels*

3 Terms and definitions

For the purposes of document, the following terms and definitions apply.

3.1
heterogeneity
measured variation in compositions of elements measured from a group of specimens

Note 1 to entry: The contributions to heterogeneity include the uncertainties in the measurements from specimen to specimen, from micrometre to micrometre within each specimen, and from the test procedure itself.

3.2
research material
material that appears to have the physical and chemical characteristics required of a CRM, but which is to be examined in detail, including the determination of chemical composition, stability, and micro-heterogeneity and macro-heterogeneity, before certification as a CRM

3.3
stability
<general>resistance of a specimen to chemical and physical change during long-term storage at normal temperature and pressure

3.4
stability
<EPMA>resistance of the material to changes in chemical composition during electron bombardment, i.e. the resistance to change of the intensity of the relevant characteristic X-rays observed during the time the specimen is exposed to the electron beam

3.5
uncertainty
quantitative statement that provides a value for the expected deviation of a measurement from an estimate of the value of the specific measured quantity

4 Preparation of the research material

4.1 Selection of material

The research material used for the preparation of a CRM should exhibit little or no heterogeneity on a micrometre scale, should be free from unwanted inclusions, and should be sufficiently dense (such that voids, if present, can be readily avoided during testing and analysis) and stable under prolonged electron bombardment.

The mounted research material should be of sufficient size to provide several areas suitable for point beam analysis; each area should be approximately 20 µm or more in diameter. At a minimum, the size should be at least twice the area of X-ray emission.

The quantity of research material should be adequate for the preparation of certified specimens.

In the case of a synthetic RM, a detailed description of the preparation technique should be provided. In the case of minerals, the geographic origin, the source, and the separation process should be specified.

4.2 Preliminary inspection of the material

Initial inspection of a possible research material for a CRM should be made using a binocular optical microscope to evaluate the material for the presence of unwanted inclusions, voids, or other phases, and if these are found to be sufficiently abundant to interfere with EPMA of the major phase of interest, i.e. to prevent a clean sampling of the major phase at multiple points with a 1 µm electron beam, the material should be rejected.

Further inspection for the possible presence of very small inclusions or other phases should be carried out on polished sections in reflected and/or transmitted light. An electron microprobe or a scanning electron microscope with secondary electron and backscatter electron detectors might be needed. Material of known composition with inclusions or other phases should only be considered suitable if the inclusions or other phases can be easily identified and clearly marked on accompanying documentation so that they can be avoided during use.

Material found suitable after preliminary inspection should subsequently be processed for further determination of heterogeneity and stability.

5 Heterogeneity of material

5.1 Sample preparation

The CRM should be stable under the electron beam. It should not charge under required test conditions, though in some cases, a conductive coating might be required. It should be in such a physical state that it can be mounted and polished if necessary without rapid surface deterioration on exposure to the atmosphere or vacuum.

The research material should be in the same or similar physical orientation as that proposed for the CRM, e.g. if the CRM is to be cut or cleaved so that flat surfaces are to be used by the analyst for EPMA, then the research material should be mounted in the same manner as that used to obtain heterogeneity data.

5.2 Sample size

The number of specimens selected for testing will depend upon the number, size, and composition of the individual specimens in the sample group.

For a large number of specimens, such as 200 or more seemingly identical specimens already cut or cleaved and ready for distribution, testing of all specimens would be prohibitively time consuming. A statistically representative number of randomly selected specimens should be selected for testing. If the

measured heterogeneity between and/or within specimens is observed to be greater than 1 % relative after taking account of counting statistics for the elements being certified, testing of more specimens might be needed.

Where there are fewer specimens, typically 5 to 20, which can be tested before being cut into smaller specimens for distribution, each specimen may be analysed before being cut, provided that the preparation process does not change the composition in any way.

Consultation with an experienced statistician is strongly recommended before data acquisition is begun. Detailed rules regarding the sample size are avoided here to allow the analyst flexibility in designing the testing procedures since decisions will depend upon the characteristics of the material and the number of specimens available.

5.3 Test conditions

If the extent of heterogeneity is being determined on the micrometre scale, a 1 µm (point) beam should be used for the analysis. In some cases, where there might be damage to the specimen by the electron beam, a defocused beam, typically 5 µm diameter, may be used. Such samples should, therefore, be certified for use only with a defocused beam.

Wavelength-dispersive X-ray spectroscopy (WDX) is the preferred method for heterogeneity determinations because the high X-ray peak rates obtainable with the technique expedite the acquisition of statistically useful data. Energy-dispersive X-ray spectrometry (EDX) can be applied by using integrated X-ray peak intensities, but the data acquisition process is significantly longer. For specimens sensitive to the high current needed for WDX, EDX can be the only choice.

Ideally, the excitation voltage used for the analysis should be about two and a half times the critical excitation energy of the X-ray line of the element being analysed, although this can be difficult if several elements are analysed simultaneously. As a compromise, the selected excitation voltage should be sufficient to excite the X-ray lines of the elements used in the testing with an adequate overvoltage of at least 1,5 times the critical excitation potential.

The selected X-ray lines used to acquire the heterogeneity data should not overlap any X-ray lines of other elements in the specimen. This can be ascertained from wavelength dispersive spectrometer (WDS) scans of the pure elements (or appropriate well-characterized compound specimen in which overlap does not present a problem) and of the RM.

The current used will depend upon element concentrations, the stability of the specimen to the electron beam, and the count rate desired.

The count rate should provide acceptable counting statistics. The count rate should not be so high that the dead time of the WDS proportional counter will increase beyond the normal working range. A normal proportional counter dead time is 1,2 µs or less. For energy dispersive spectrometer (EDS), the dead time should be approximately 30 %.

NOTE Acceptable count rates will also depend upon tolerable counting uncertainties. From Poisson counting statistics, the standard uncertainty in the counts obtained from an X-ray measurement is equal to the square root of the total number of X-ray counts, $\sqrt{N}$. A 1 % error can be obtained when the total number of counts is 10 000, but this relative error can be reduced by increasing the number of counts. At 100 000 counts, the relative error is reduced to 0,3 %. For an EDS, the number of counts refers to the counts in the window of interest or integrated peak counts, not the total spectrum counts. This test uncertainty will be present regardless of the extent of heterogeneity and can be minimized by increasing the integral number of counts through increased current and/or counting time at a given excitation voltage. Both ultimately depend on the specimen stability, while the counting time will also be limited by test practicality.

Knowing the estimated count rate, R, and the desired relative error, σ, the counting time, T, required to achieve that relative error can be calculated from the equation $T = 1/\sigma^2 R$. This equation is derived from the Poisson estimate of the relative error due to counting statistics,

$$1/\sqrt{(N)} = 1/\sqrt{(RT)}$$

5.4 Test procedure

Before heterogeneity testing is begun, the edges of bulk specimens should be analysed and compared to the specimen interior to determine whether there might be a consistent difference in element concentrations in the two locations. Occasionally, differences can result from the manufacturing process of materials such as metal alloys or synthetic crystals. If the edges are different from the specimen interior, they should be removed before samples are taken for bulk quantitative analysis and before specimens are mounted and polished for heterogeneity studies. In some specimens, differences might also be due to mounting and polishing procedures; if this occurs and cannot be remedied, the certificate should include instructions to the analyst to avoid using the material within a specified minimum distance from the edge.

Specimens that are being compared should be mounted together in the same sample mount or block, if possible. Carbon coating, if necessary, should be applied to all specimens simultaneously.

Tests should be designed to efficiently acquire the data needed to determine the extent of the within-specimen and between-specimen heterogeneity, to determine the experimental uncertainty, and to look for gradual increasing or decreasing concentration changes on the micrometre scale using 50 µm to 100 µm line scans. Examples of tests are given below, but they may be modified depending upon the individual material or group of specimens being analysed. The beam current should be monitored to provide a value corresponding to each data reading enabling subsequent current drift corrections to be carried out, if necessary.

NOTE It is advisable to collect data in an ASCII format that can be easily put into a spreadsheet for subsequent processing.

For each specimen being tested, X-ray counts for several, randomly selected points (typically 7 to 10 or more depending upon the size of the specimen) should be acquired. These data should be acquired at least in duplicate i.e. integral X-ray counts should be acquired and recorded at least twice on each point without moving the specimen or electron beam between acquisitions. Specimens should be analysed in a random order and preferably, each specimen should be analysed twice, each time in a different order. It may be worthwhile for different operators to take data for duplicate analyses, using a different random sampling plan for each. Refer to ISO Guide 35[6] for sampling procedures and methods of evaluating results. The data from this type of test is used to calculate the within-specimen and between-specimen uncertainties, as well as the test uncertainty after beam current drift corrections are made. When background data are obtained for each element, the uncertainties can be expressed as a mass fraction. The formulae used for these calculations are given in 5.5.

To test for the presence of concentration trends within each specimen, which might not be detected by random sampling, line profiles of the points less than 5 µm apart and 50 µm to 100 µm in length should be prepared. Two-line profiles normal to one another are recommended. For specimens of 1 cm to 2 cm, a set of two-line profiles should be prepared from at least two different locations on the specimen. After current corrections, data should be plotted (distance against X-ray counts) for each element to expose variations in concentrations that might be present. Such trends might not preclude the certification process if they are within the 99 % confidence limits or ±3 times the Poisson counting error (square root of the integral number of X-ray counts).

5.5 Statistical evaluation of data

The uncertainties in the element concentrations resulting from heterogeneity within specimens and between specimens and in the test acquisition can be obtained from the procedures described above using the following calculations.

NOTE There are several examples[1][2][3][4] of the use of test procedures and calculations similar to those described here; the statistical notation has been simplified for this document to facilitate its usage. The statistical approach used here is called a nested design that is described in detail in other references.[5][6][8] The procedures described have been developed in collaboration between the National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA and the National Physical Laboratory (NPL), Teddington, Middlesex, UK and have been used successfully. Other validated test and statistical procedures may be used, provided that they are described in full in the CRM certificate.

Let w_0 be the true mass fraction of a particular element in the RM. Any single micrometre scale measurement, w, expressed in weight percent taken from a randomly selected point of a randomly selected specimen will deviate from w_0 because of the variation between specimens (macroheterogeneity), variation within specimens (microheterogeneity), and the measurement error. The deviation, $w - w_0$, may be viewed as a sum of random effects, as shown in Formula (1):

$$w = w_0 + \mathrm{S} + \mathrm{P} + \mathrm{E} \tag{1}$$

where

$w_0 + \mathrm{S}$ is the true mass fraction in the selected specimen;

$w_0 + \mathrm{S} + \mathrm{P}$ is the true micrometre scale mass fraction concentration at the selected point of the selected specimen;

E is the measurement error.

The components of variance $\sigma^2_{\mathrm{S}_w}$, $\sigma^2_{\mathrm{P}_w}$, and $\sigma^2_{\mathrm{E}_w}$ are the variances of the random effects S, P, and E, respectively. The variance, σ^2_w, of the measurement w is given by Formula (2):

$$\sigma^2_w = \sigma^2_{\mathrm{S}_w} + \sigma^2_{\mathrm{P}_w} + \sigma^2_{\mathrm{E}_w} \tag{2}$$

If n_{E} independent measurements are made at each of n_{P} randomly selected points of each of n_{S} randomly selected specimens and if w_{ijk} denotes the kth replicated measurement at point j of specimen i, then the grand mean given by Formula (3):

$$\bar{w} = \frac{1}{\left(n_{\mathrm{P}}\ n_{\mathrm{S}}\ n_{\mathrm{E}}\right)} \sum_{i=1}^{n_{\mathrm{S}}} \sum_{j=1}^{n_{\mathrm{P}}} \sum_{k=1}^{n_{\mathrm{E}}} w_{ijk} \tag{3}$$

has a variant, given by Formula (4):

$$\sigma^2_{\bar{w}} = \frac{\sigma^2_{\mathrm{S}_w}}{n_{\mathrm{S}}} + \frac{\sigma^2_{\mathrm{P}_w}}{n_{\mathrm{S}} n_{\mathrm{P}}} + \frac{\sigma^2_{\mathrm{E}_w}}{n_{\mathrm{S}} n_{\mathrm{P}} n_{\mathrm{E}}} \tag{4}$$

assuming the design is balanced. Thus, the uncertainty in the mean measurement $\bar{w}$ can be determined from estimates of $\sigma^2_{\mathrm{S}_w}$, $\sigma^2_{\mathrm{P}_w}$, and $\sigma^2_{\mathrm{E}_w}$. An approximate 95 % or 99 % confidence interval for the mean micrometre scale concentration is respectively

$$\bar{w} \pm 2\left[\frac{\sigma^2_{\mathrm{S}_w}}{n_{\mathrm{S}}} + \frac{\sigma^2_{\mathrm{P}_w}}{n_{\mathrm{S}} n_{\mathrm{P}}} + \frac{\sigma^2_{\mathrm{E}_w}}{n_{\mathrm{S}} n_{\mathrm{P}} n_{\mathrm{E}}}\right]^{1/2} \tag{5A}$$

or

$$\bar{w} \pm 3\left[\frac{\sigma^2_{\mathrm{S}_w}}{n_{\mathrm{S}}} + \frac{\sigma^2_{\mathrm{P}_w}}{n_{\mathrm{S}} n_{\mathrm{P}}} + \frac{\sigma^2_{\mathrm{E}_w}}{n_{\mathrm{S}} n_{\mathrm{P}} n_{\mathrm{E}}}\right]^{1/2} \tag{5B}$$

Estimates of $\sigma^2_{\mathrm{S}_w}$, $\sigma^2_{\mathrm{P}_w}$, and $\sigma^2_{\mathrm{E}w}$ can be obtained from the raw count data as follows.

Let Y_{ijk} denote the kth count measured at point j of specimen i, and let B_{ijk} represent the background count associated with the measured count Y_{ijk}. Assuming a linear relationship between the number of