A MODIFIED APPROACH TO HOMOGENEITY TESTING AT MICROSCALE

Marco Mattiuzzi and Andrzej Markowicz

IAEA Laboratories Seibersdorf
A-2444 Seibersdorf, Austria

Abstract

A modified approach to homogeneity testing of reference materials for microanalytical techniques, based on the concept of the information mass and the prescription of homogeneity testing for bulk material (sample mass >100 mg), is presented. A microanalytical instrumental set-up is used to study homogeneity at mass level below 1 mg and a modified version of T and F statistical tests is applied. The sensitivity of the method to identify contaminated materials is assessed with Monte Carlo simulations and a good accuracy in the identification of contaminants is demonstrated. As an example of the application of the approach the analysis of the IAEA-SL1 reference material is performed and the homogeneity test shows homogeneous distribution of most of the elements for sub µg masses. For these elements the calculation of the mass levels required for a desired accuracy is presented. A method to determine the mass level for homogeneous distribution for the remaining elements identified is also suggested.

Introduction

From the ISO definition \cite{1} of homogeneity “The condition of being of uniform structure or composition with respect to one or more specified properties. A reference material is said to be homogenous with respect to a specified property if the property value, as determined by tests on samples of specified size, is found to lie within the specified uncertainty limits, the sample being taken either from a different supply units (bottles, packages, etc.) or from a single supply unit” some key concepts can be extracted: 1) homogeneity is a fundamental property of reference materials, it assures uniformity in structure or composition, 2) homogeneity is not an overall or general quality factor but is related to one or more specified properties of the material, 3) the homogeneity assessment is done through statistical tests giving uncertainty limits; 4) homogeneity is always linked to a specified sample mass/size, and finally 5) homogeneity does not depend on the source (supply unit) of the sample analyzed. Summarizing, a practical meaning that embodies the mentioned requirements can be given: the result of analysis for a homogeneous material should not depend on which part of the material is selected.

For the homogeneity certification/assessment statistical tests are conducted on the results obtained for different parts of the material in order to verify that the same properties are observed. A few samples are taken at random from one supply unit/bottle and from different supply units/bottles, as described in Thompson and Wook \cite{2}, next the analysis of variance at the α significance level is performed. In Dybczyński et al.\cite{3} a different approach in the statistics applied is suggested. The mean values and standard deviations are calculated and T and F statistical tests at the significance level α are done, respectively. In both approaches the tests are repeated for every identified element.
This document was presented at the Denver X-ray Conference (DXC) on Applications of X-ray Analysis.

Sponsored by the International Centre for Diffraction Data (ICDD).

This document is provided by ICDD in cooperation with the authors and presenters of the DXC for the express purpose of educating the scientific community.

*All copyrights for the document are retained by ICDD.*

Usage is restricted for the purposes of education and scientific research.

DXC Website
– [www.dxcicdd.com](http://www.dxcicdd.com)

ICDD Website
- [www.icdd.com](http://www.icdd.com)
For bulk analysis the reference materials are commonly certified for sample masses of the order of 100 mg. The analytical techniques that investigate the material composition below this limit obviously require a suitable standard material for calibration and quality control.

It is important to discern the difference between a material studied at bulk level and at microscale. All materials are composed of discrete constituents. In bulk analysis a large number of these building blocks is involved and potential heterogeneities are reduced through the averaging effect. In microanalysis the sample mass is reduced, the averaging becomes less effective and the heterogeneities more evident. The difference then lies in the “degree of magnification” at which material is observed; the smaller the mass sampled the coarser the sample appears to the analyst. This effect is reflected in the increase of the ratio of the standard deviation (free from analytical error) to the mean value (relative standard deviation, RSD) as the mass of sample diminishes. To characterize the material, the sampling constant \( k \) was introduced, Ingamells and Switzer\(^4\) Kurfürst, Grobecker and Stoeppler\(^5\); it relates the RSD and the sample mass through \( \text{RSD} = k / (m)^{1/2} \).

As the meaning of homogeneity does not change with mass level the ideal homogeneity assessment should be conducted at the lowest mass level. The analysis of small masses cannot be done with the analytical techniques for bulk materials and a different instrumental set-up is required. Various analytical methods were applied for the homogeneity assessment at microscale, e.g., solid sampling Zeeman effect AAS at mg level, Sonntag and Rossbach\(^6\) and EPMA single particle analysis at ng level, Hoornaert et al\(^7\).

A new method is presented in this paper which is based both on the information mass concept and on modified statistical test procedures. With this approach the homogeneity assessment for all microanalytical techniques, including \( \mu \)-XRF, \( \mu \)-PIXE, \( \mu \)-SRXRF, EPMA... can be standardized. The method follows a general idea of homogeneity testing at bulk level, and permits the assessment of homogeneity at mass scale from ng up to some mg.

**Homogeneity assessment in general**

The starting point for the proposed microanalytical method for homogeneity testing is determined by the protocol used for bulk material. Candidate reference materials are usually divided into supply units or bottles.

![Fig. 1 Schematic representation of the sampling procedure for homogeneity testing](image)
According to Thompson and Wook and Dybczyński et al., the homogeneity assessment requires that some random samples are measured. Some samples (M ≥ 2) are picked at random from a random bottle and measured; other samples (10 ≤ n ≤ N) are taken from random bottles and measured (see Fig. 1). Statistical analysis is then performed on the two sets of results which create the in-bottle and the between-bottle distributions (IB and BB respectively). In this way the tests can provide evidence that the results do not depend on the choice of the bottle/s the samples are taken from.

The mass level at which the homogeneity assessment is performed is given by the information mass, IM. The information mass is the mass of the material that gives 100% of the measured signal, e.g., intensity of the characteristic X-rays. The concept can simply be visualized in Fig. 2, right part.

Fig. 2 Schematic representation of a scan on one pellet of a candidate reference material. On the left side the top view of the pellet with NxM small dots representing the analyzed pixels. On the right side the blow up of only one pixel with a perspective view of the incident beam of spot size S, a pellet with thickness D and the penetration depth d of the radiation.

In case a beam of spot size S impinges on a pellet of thickness D then only a fraction of the material within the cylindrical volume d·S contributes to the measured signal, where d is the penetration depth of the radiation. For the thick pellet (D > d), the information mass IM is given by equation 1, with \( d^* = d \cdot \rho_t \) [g/cm²] where \( \rho_t \) is the density of the material.

\[
IM = S \cdot d^* = S \cdot m_{thick} \quad (1)
\]

The information mass IM depends on the energy of the X-rays through \( m_{thick} \) (different information masses are associated to different elements), and on the primary photon beam spot size S. Typical information masses for bulk analysis, where \( S \approx 1 \text{ cm}^2 \), are about 100 mg.

**Method**

The new method has the following differences compared to that applied for the bulk samples: i) sub ng mass level can be investigated by focusing/reducing beam sizes to sub μm range, ii) a
single pellet with 1 cm in diameter can be scanned and a large number of pixels, $10^5-10^6$, can be analyzed, iii) a modified statistical analysis protocol is needed to handle numerous populations. The method proposed include the following steps:

1. Prepare a thick pellet
2. Scan the pellet in NxM pixels (see Fig. 2, left part)
3. Store on a pixel basis the intensities of the characteristic X-ray lines for the identified elements
4. For each element divide the results into N “bottles” (vertical scan) each containing M results.

This procedure allows the collection of a great number of results (up to $10^5-10^6$). The statistical analysis of the results is structured as follows (see also Table 1):

1. Select at random $m < M$ results from generic “bottle” $1 < j < N$ (IB population)
2. Select at random N results, taking one result from each of the N “bottles” (BB population)
3. Do T and F tests on the two populations selected in point 1 and 2
4. Repeat the process described in 1, 2, 3 for $10^2$ times
5. Repeat the process described in 4 for more than $10^2$ times.

<table>
<thead>
<tr>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>B5</th>
<th>B6</th>
<th>B7</th>
<th>B8</th>
<th>B9</th>
<th>B10</th>
<th>B11</th>
<th>B12</th>
<th>B13</th>
<th>B14</th>
<th>B15</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>1157</td>
<td>1334</td>
<td>1176</td>
<td>1345</td>
<td>1204</td>
<td>1349</td>
<td>1341</td>
<td>1464</td>
<td>1147</td>
<td>1391</td>
<td>1002</td>
<td>1252</td>
<td>1131</td>
<td>1224</td>
</tr>
<tr>
<td>S2</td>
<td>1257</td>
<td>1312</td>
<td>1262</td>
<td>1145</td>
<td>1359</td>
<td>1305</td>
<td>1235</td>
<td>1207</td>
<td>1287</td>
<td>1064</td>
<td>1521</td>
<td>1291</td>
<td>1235</td>
<td>1275</td>
</tr>
<tr>
<td>S3</td>
<td>1277</td>
<td>1267</td>
<td>1270</td>
<td>1290</td>
<td>1271</td>
<td>1155</td>
<td>1429</td>
<td>1494</td>
<td>1293</td>
<td>1349</td>
<td>1406</td>
<td>1459</td>
<td>1219</td>
<td>1518</td>
</tr>
<tr>
<td>S4</td>
<td>1232</td>
<td>1432</td>
<td>1211</td>
<td>1422</td>
<td>1124</td>
<td>1280</td>
<td>1283</td>
<td>1296</td>
<td>1213</td>
<td>1298</td>
<td>1707</td>
<td>1174</td>
<td>1376</td>
<td>1500</td>
</tr>
<tr>
<td>S5</td>
<td>1474</td>
<td>1239</td>
<td>1325</td>
<td>1316</td>
<td>1187</td>
<td>1273</td>
<td>1164</td>
<td>1081</td>
<td>1659</td>
<td>1405</td>
<td>1195</td>
<td>1499</td>
<td>1380</td>
<td>1337</td>
</tr>
<tr>
<td>S6</td>
<td>1362</td>
<td>1341</td>
<td>1219</td>
<td>1323</td>
<td>1214</td>
<td>1377</td>
<td>1389</td>
<td>1225</td>
<td>1244</td>
<td>1292</td>
<td>1113</td>
<td>1640</td>
<td>1365</td>
<td>1452</td>
</tr>
<tr>
<td>S7</td>
<td>1205</td>
<td>1492</td>
<td>1400</td>
<td>1088</td>
<td>1364</td>
<td>1564</td>
<td>1409</td>
<td>1224</td>
<td>1295</td>
<td>1336</td>
<td>1323</td>
<td>1335</td>
<td>1315</td>
<td>1429</td>
</tr>
<tr>
<td>S8</td>
<td>1361</td>
<td>1287</td>
<td>1272</td>
<td>1319</td>
<td>1450</td>
<td>1403</td>
<td>1212</td>
<td>2155</td>
<td>1753</td>
<td>1366</td>
<td>1318</td>
<td>1319</td>
<td>1317</td>
<td>1253</td>
</tr>
<tr>
<td>S9</td>
<td>1380</td>
<td>1367</td>
<td>1191</td>
<td>1304</td>
<td>1359</td>
<td>1442</td>
<td>1327</td>
<td>1382</td>
<td>1170</td>
<td>1327</td>
<td>1445</td>
<td>1472</td>
<td>1419</td>
<td>1381</td>
</tr>
<tr>
<td>S10</td>
<td>1159</td>
<td>1197</td>
<td>1309</td>
<td>1292</td>
<td>1374</td>
<td>1233</td>
<td>1887</td>
<td>1250</td>
<td>1533</td>
<td>1162</td>
<td>1216</td>
<td>1234</td>
<td>1443</td>
<td>1296</td>
</tr>
<tr>
<td>S11</td>
<td>1357</td>
<td>1376</td>
<td>1352</td>
<td>1254</td>
<td>1452</td>
<td>1276</td>
<td>1168</td>
<td>1302</td>
<td>1499</td>
<td>1400</td>
<td>1387</td>
<td>1253</td>
<td>1234</td>
<td>1665</td>
</tr>
<tr>
<td>S12</td>
<td>1394</td>
<td>1360</td>
<td>1374</td>
<td>1101</td>
<td>1426</td>
<td>1209</td>
<td>1355</td>
<td>1401</td>
<td>1388</td>
<td>1340</td>
<td>2924</td>
<td>1334</td>
<td>1316</td>
<td>1403</td>
</tr>
<tr>
<td>S13</td>
<td>1283</td>
<td>1400</td>
<td>1174</td>
<td>1482</td>
<td>1241</td>
<td>1457</td>
<td>1288</td>
<td>1395</td>
<td>1283</td>
<td>1300</td>
<td>1451</td>
<td>1408</td>
<td>1408</td>
<td>1277</td>
</tr>
<tr>
<td>S14</td>
<td>1391</td>
<td>1284</td>
<td>1318</td>
<td>1151</td>
<td>1238</td>
<td>1306</td>
<td>1292</td>
<td>1372</td>
<td>1425</td>
<td>1590</td>
<td>1180</td>
<td>1314</td>
<td>1166</td>
<td>1348</td>
</tr>
<tr>
<td>S15</td>
<td>1311</td>
<td>1417</td>
<td>1246</td>
<td>1303</td>
<td>1417</td>
<td>1192</td>
<td>1137</td>
<td>1291</td>
<td>1334</td>
<td>1411</td>
<td>1288</td>
<td>1281</td>
<td>1277</td>
<td>1741</td>
</tr>
</tbody>
</table>

Table 1 Example of data arrangement for the proposed statistical analysis. The table contains the intensities measured for one element in a 15x15 scan. Columns represent 15 “bottles” (B1..B15). Each column contains the results of measurements for 15 samples (S1..S15). A random choice of 7x15, IBxBB population, is shown by underlined and bold figures, respectively. The selection (see highlighted figures) is one out of $10^{22}$ possible combinations.

T and F statistical tests are used for the homogeneity assessment as suggested in Dybczyński et al.\cite{3}. The probability of passing T and F tests, $P_{T\&F}$, is then calculated over the whole cycle, points 1 to 5 in the scheme above. The associated uncertainty/error is computed storing the partial probabilities every 100 steps, points 1 to 4 in the scheme above.

Based on the definition of the significance level $\alpha$, Natrella\cite{9}, the probability of passing both tests for homogeneous materials is $P_{T\&F} = 1 - \alpha_T - \alpha_F + \alpha_T \alpha_F$, where $\alpha_T$ and $\alpha_F$ are the significance levels for T and F tests, respectively. A candidate reference material is not
homogeneous for a certain analyte if the calculated probability (with the associated uncertainty) fulfills equation 2.

\[ P^{T&F} < 1 - \alpha_T - \alpha_F + \alpha_T \alpha_F \quad (2) \]

The individual probabilities for T or F tests separately can be calculated likewise. If \( P^T < 1 - \alpha_T \) or \( P^F < 1 - \alpha_F \) the material is considered heterogeneous only for the mean or the standard deviation respectively. \( P^{T&F} \) can also be calculated with \( P^{T&F} = P^T \cdot P^F \).

**Sensitivity**

In order to assess the sensitivity of the homogeneity test, a Monte Carlo simulation was performed. A numerical model was created to simulate the measurement process of a generic analyte distributed homogeneously and heterogeneous in two materials. The intensity of the characteristic X-rays, \( H \), for the generic element has different distribution for the two materials. For the homogeneous case the results are scattered around the true/homogeneous value \( H_\sigma \) with a standard deviation \( \sigma \) that was initially assumed to be equal \((H_\sigma)^\frac{1}{2}\) (see Fig. 3 left). The method was applied to the population of the results following the procedure described in points 1-5 in the previous section. The initial value of the standard deviation was varied from \( \sigma \) to \( 30 \sigma \) and the computed probability of passing the test \( P^{T&F} \) was always \( \geq 1 - \alpha_T - \alpha_F + \alpha_T \alpha_F \). The heterogeneous material was modeled by adding a “contaminant” to the homogeneous material, centered at \( H_c = H_\sigma + n \sigma \); the probability of contamination is \( P_c \). The standard deviation for the contaminant distribution is calculated from \( \sigma_e = (H_c)^\frac{1}{2} \).

![Fig. 3 Distributions of the simulated intensity P(H) for a generic analyte in a homogeneous (left) and heterogeneous material (right). For both cases solid curves represent the homogeneous distribution; the dashed curve on the right figure shows the distribution of a contaminant defined by the parameters Hc and Pc.](image)

Letting the parameter \( n \) vary from 2 up to 11 and \( P_c \) from 0.025 up to 0.25 the response of the method to the different types of contamination was tested. The results are presented in Fig. 4 (the uncertainties on individual points (± 0.02) are not reported). The probability of passing T and F test, \( P^{T&F} \), can be calculated as a product of \( P^T \cdot P^F \). The curves in Fig. 4 show a good sensitivity of the method to identify contaminated materials; small contamination can be detected at 0.025 contamination probability.
Application to a candidate reference material for microanalytical techniques

The method for testing homogeneity at microscale was applied to a reference material, IAEA-SL1 (Lake Sediment) which was already certified at 100 mg level with elemental concentrations ranging from 6 ppm for Br up to 6% for Fe \(^\text{[10]}\).

Fig. 4 Probability of passing $T$ (left side) and $F$ (right side) statistical tests as function of degree of contamination (defined by the parameter $n$) for different contamination probability. Joint probability $P_p F$ is the product of the two probabilities obtained for a given set of the parameters $H_e, P_e$.

Fig. 5 Probability of passing $T$ (top) and $F$ (bottom) test at significance level $\alpha=0.05$ for the identified elements in the IAEA-SL1 reference material. The solid line represents the borderline for homogeneity. If the bars+errors are above this limit for both $T$ and $F$ tests the elements are considered homogeneously distributed.
A thick pellet of the material was prepared and a scan of 15x15 pixels was done by using the micro XRF set-up described in Bernasconi et al.\textsuperscript{[11]}. The sample was at 90° relative to capillary axis; the detector was at 45° relative to the sample. Mo X-ray tube was used at 50 kV and 30 mA; the measuring time per pixel was 500 s. The distances capillary-sample and sample-detector were 2 and 12 mm, respectively. The information mass (with a beam spot area $S = 700 \mu m^2$, 10 $\mu m$ capillary) was roughly between 0.1 and 1 $\mu g$ for the characteristic X-rays in the energy range of 3-14 keV. The method described was applied for all the identified elements. The probabilities of passing the 'I' and F tests ($P^I$, $P^F$ respectively) were calculated for 7x15 (IBxBB) population and $10^4$ random choices (results are reported in Fig. 5). The material is said to be homogeneous for the generic analyte if both the probabilities are not below the 0.95 borderline ($\alpha_I = \alpha_F = 0.05$), within the error bar. The RSD calculated for the elements distributed homogeneously in the IAEA-SL1 are given in Table 2.

<table>
<thead>
<tr>
<th>Element</th>
<th>Cr</th>
<th>Co</th>
<th>Ni</th>
<th>Cu</th>
<th>Ga</th>
<th>Br</th>
<th>Rb</th>
<th>Sr</th>
<th>Ba</th>
<th>Gd</th>
<th>W</th>
<th>Au</th>
<th>Pb</th>
<th>Th</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSD %</td>
<td>18</td>
<td>12</td>
<td>125</td>
<td>19</td>
<td>20</td>
<td>68</td>
<td>7</td>
<td>12</td>
<td>56</td>
<td>37</td>
<td>32</td>
<td>40</td>
<td>11</td>
<td>32</td>
</tr>
</tbody>
</table>

Table 2 Measured relative standard deviations for different elements in the IAEA-SL1 reference material (at 1 pixel level)

The results refer to the information mass reported in Fig. 6, as a function of the X-ray energy. The RSD values are high for most of the elements except Co, Rb, Sr and Pb (below 15%). Using the sampling constant ($k$) concept the following formula for the RSD$_M$ measured at mass $M=nm_o$ is derived

$$RSD_M = RSD_o / \sqrt{n}$$  \hspace{1cm} (3)

where $RSD_o$ is measured at the mass $m_o$. Based on eq. 3 one can assess the mass level at which the homogeneity of the analyte of interest is characterized by a given value of RSD. In Table 3 the masses which correspond to 5% relative standard deviation are presented. As an example, the information mass for one pixel in case of Cl (5.4 keV X-ray) is roughly 0.12 $\mu g$. If the information mass for the analyte is higher than $M = 0.12 \cdot 13 = 1.5\mu g$ the RSD becomes smaller than 5%.

<table>
<thead>
<tr>
<th>Element</th>
<th>Cr</th>
<th>Co</th>
<th>Ni</th>
<th>Cu</th>
<th>Ga</th>
<th>Br</th>
<th>Rb</th>
<th>Sr</th>
<th>Ba</th>
<th>Gd</th>
<th>W</th>
<th>Au</th>
<th>Pb</th>
<th>Th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass Lev.</td>
<td>13</td>
<td>6</td>
<td>628</td>
<td>14</td>
<td>17</td>
<td>183</td>
<td>2</td>
<td>6</td>
<td>123</td>
<td>55</td>
<td>42</td>
<td>65</td>
<td>5</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 3 Mass levels (expressed as multiples of the information mass for 1 pixel, see Fig. 6) at which the homogeneity of the distribution for the identified elements, defined by RSD, is equal to 5%\textsuperscript{2}.

The method can also be used to assess homogeneity at different mass levels. Taking into account that the information mass for one pixel corresponds to IM, an assessment of homogeneity at any mass level $M^* = m IM$ can be done in the following way. The results for m pixels are summed and a new population with NxM/m representatives is created, the statistical analysis is performed as before. This approach is particularly useful for those elements that show heterogeneous distribution at the level of a single pixel (1 IM), as e.g., for Cl, K, Ti, Fe, Mn, Zn and Sb in case of the IAEA-SL1 reference material. The homogeneity was performed at IM$^* = 3$ IM and the results showed that all the elements are heterogeneously distributed also at this level.
Conclusions

A modified approach to test the homogeneity of the materials at microscale was proposed. The method is applicable to all microanalytical techniques, including μ-XRF, μ-SXRF, μ-PIXE and EPMA that can easily provide a large population of results (up to 10^5). The major advantages of the method can be summarized as follows: i) a very simple sample preparation is required and uncertainties introduced by this step are minimized, ii) a wide range of mass levels, from ng up to mg, can be investigated simply by changing the beam spot size, iii) numerous populations are produced in a short time, iv) local analysis is performed. In order to fully exploit these advantages a specific statistical protocol was developed. Its main characteristics are: I) a huge amount of results are processed in an automatic and flexible way, all data are treated via software and there is no limitation but computer time, II) topological information from the local microanalysis is preserved, populations are divided into sub-groups which correspond to defined areas on the sample, III) standard statistical tests are used, IV) good sensitivity to identify the contaminants (as demonstrated with Monte Carlo simulations) V) mass level of the material for a given relative standard deviation of the elemental distribution and for homogeneous distribution can be predicted. This method, combined with any microanalytical technique, can be used for the homogeneity test of candidate reference material at any mass level.

REFERENCES


