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Reference materials for microanalytical nuclear techniques

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FOREWORD

In 1994 the IAEA established a Co-ordinated Research Project (CRP) on Reference Materials for Microanalytical Nuclear Techniques as part of its efforts to promote and strengthen the use of nuclear analytical technologies in Member States with the specific aim of improving the quality of analysis in nuclear, environmental and biological materials. The objectives of this initiative were to:

- identify suitable biological reference materials which could serve the needs for quality control in micro-analytical techniques,
- evaluate existing CRMs for use in micro-analytical investigations,
- evaluate appropriate sample pre-treatment procedures for materials being used for analysis with micro-analytical techniques,
- identify analytical techniques which can be used for characterisation of homogeneity determination, and
- apply such techniques to the characterization of candidate reference materials for use with micro-analytical techniques.

The CRP lasted for four years and seven laboratories and the IAEA's Laboratories in Seibersdorf participated. A number of materials including the candidate reference materials IAEA 338 (lichen) and IAEA 413 (single cell algae, elevated level) were evaluated for the distribution of elements such as Cl, K, Ca, Cr, Mn, Fe, Zn, As, Br, Rb, Cd, Hg and Pb.

The results obtained during this CRP suggest that i) each element exhibits its characteristic distribution in a matrix described by the "Ingamels sampling constant" or the "relative homogeneity factor" of Kurfuerst, ii) both concepts are valid over a large range of sample mass used for analysis (from 0.1 µg to around 100 mg) and iii) materials being characterised quantitatively for elements homogeneity could be used for the experimental determination of total uncertainty of other analytical techniques.

The first research co-ordination meeting (RCM) was held from 13 to 16 December 1994 at the Rudjer Boskovic Institute, Zagreb, Croatia. The participants introduced their respective analytical techniques in working papers. They also emphasised, which of the technique's properties may make the procedures particularly suitable for the characterisation of small samples. The sample size capabilities of the presented techniques ranged from ultra micro samples of nanogram sample mass to macro samples in the larger than 100 mg range. Also introduced to the CRP were tests on several sample types of biological and environmental samples that demonstrated some potential for these materials for use with microanalytical techniques.

The second RCM was organised by the Institute of Physics, UNAM, Mexico City, Mexico, from 30 May to 5 June 1996. The participants presented results obtained with their techniques on the AQCS test materials of urban particulate matter, IAEA 396A/S and 396A/M, including results from the newly introduced technique of computer controlled electron probe X ray microanalysis (EPXMA). The participants identified opportunities to further develop the applicable analytical techniques, to introduce new matrices in this suite of new CRMs, and to research fundamental parameters affecting homogeneity in the analytical investigations of small samples.

The final RCM was held in Vienna from 7 to 11 December 1998. The status of the investigations is described in this TECDOC, including the significant beneficial outcome of this CRP demonstrating a significant improvement of the participants' technical capabilities. Results were presented that confirm the suitability of the lichen test material IAEA 338 (11 elements) and the algae test material (13 elements) which can be considered homogeneous enough for use in microanalytical calibration.

The IAEA officers responsible for this publication were M. Rossbach of the Division of Physical and Chemical Sciences and E. Zeiller of the Agency's Laboratories, Seibersdorf.

EDITORIAL NOTE

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ANNEXES

1. INTRODUCTION

In 1994 the IAEA established a co-ordinated research project (CRP) on Reference Materials for Microanalytical Nuclear Techniques, as part of its efforts to promote and strengthen the use of nuclear analytical technologies in Member States with the specific aim of improving the quality of analyses in nuclear, environmental and biological materials.

1.1 Scientific background

Instrumental trace element analysis techniques using solid samples are gaining ever broader utility in the determination of natural and pollutant constituents in biological and environmental materials. The techniques feature increased sensitivities as well as capabilities to use smaller sample sizes with the advantage of reduction or elimination of labor-intensive sample preparation processes including the chemical operations which may generate undesirable chemical waste. They are used for the determination of element concentrations in the bulk sample or the distribution of the element concentration in smaller portions of the bulk sample as well as, in combination with other techniques, for the determination of chemical species. These new techniques not only include those that through their physical principles predominantly characterize solid samples, such as X ray fluorescence, proton induced X ray emission (including their application in microprobes), and instrumental neutron activation analysis, but also many other techniques that were developed using sample dissolution and now have capabilities for solid sample introduction, including atomic absorption spectrometry and inductively-coupled plasma optical emission and mass spectrometry. Other probe techniques, such as spark source and laser ablation mass spectrometry, electron- and ion-microprobe X ray emission spectrometry, etc., have also found applications in biological and environmental studies.

A significant problem in the use of the solid- and small-sample techniques is a general lack in suitable certified reference materials (CRMs). Not only is the diversity of reference materials limited and closely matched matrix samples are not always available to test the matrix effect on a technique's accuracy, but essentially no CRMs are certified for the small sample sizes typically used. Direct utilization of most existing CRMs in solid sampling analysis procedures, i.e. analyses of samples having masses considerably smaller than 100 mg, more typically 1 mg, is often difficult or even impossible because trace components may not be sufficiently homogeneously distributed in the sample or their homogeneous distribution at this sample size has not been tested.

1.2 Objectives of the CRP

To explore the production, characterisation and use of CRMs for determinations with sample sizes much smaller than currently used, this IAEA CRP focused on the following core objectives:

- selection of the type of environmental and biological materials that will be suitable for microanalytical techniques;
- definition of specifications for suitable CRMs;
- evaluation of existing CRMs for use with microanalytical techniques;
- evaluation of requirements for sample pre-treatment such as sieving, blending, crushing, milling, drying processes and homogeneity testing;
- evaluation of analytical techniques and research on the development of techniques to be used in the characterisation of the homogeneity and chemical composition of small samples;
- application of analytical techniques to the characterisation of candidate reference materials for use with microanalytical techniques.

1.3 Development of the CRP

The CRP was established in 1994 in view of the Agency's engagement (through its Department of Technical Co-operation) in many Member States to create trace element analytical capabilities based on energy-dispersive X ray fluorescence (EDXRF), a promising low cost direct analytical technique for the assay of nuclear, environmental and biological problems. In addition, there has been growing interest in institutions of developing Member States to apply more advanced microanalytical procedures that are already available, such as particle-induced X ray emission analysis (PIXE), to measurements in environmental and biological materials and in agro-industrial products.

1.4 Nomenclature

The present CRP has been devoted to reference materials for "microanalytical nuclear techniques". One has to have in mind that the word "microanalysis" as well as "microchemistry" was not always unequivocally understood. Some clarification is given in Table I:

Table I: Magnitude of analysis expressed as size of sample used, from: *(Grant & Hackh's Chemical Dictionary, Fifth Edition, Mc Graw Hill, New York, 1987)*

Magnitude name	**Size (g)**
Macroanalysis	more than 0.1
Mesoanalysis (semimicroanalysis)	0.1–0.01
Microanalysis	10^{-2}–10^{-3}
Submicroanalysis	10^{-3}–10^{-4}
Ultramicroanalalysis	less than 10^{-4}

In the CRP and in this TECDOC the term "microanalytical methods" or "microanalytical techniques" was used for all procedures that extract analytical information from samples weighing 10 mg or less.

1.5 Homogeneity of components in materials

In discussing homogeneity properties of reference materials one has to consider some basic arguments:

- All naturally occurring materials are heterogeneous by nature as this universe is not at all well mixed (entropy is steadily increasing).
- The distribution of elements (and compounds) in a given material to some degree is random and it can be described by statistical means.
- The degree of heterogeneity of a property in a given material can be determined by repetitive measurements of the property in a number of independent units with a method of a sufficiently high degree of precision.
- According to ISO Guide 35: "material is perfectly homogeneous with respect to a given characteristic if there is no difference between the value of this characteristic from one part (unit) to another. In practice a material is accepted to be homogeneous with respect to a given characteristic if difference between the value of this characteristic from one part (or unit) to another cannot be detected experimentally. The practical concept of homogeneity therefore embodies both a specificity to the characteristic and a parameter of measurement (usually the standard deviation) of the measurement method used, including the defined sample size of the test portion" [1].

From the above general statements some important implications for the characterisation of certified reference materials follow:

1. Homogeneity statements cannot be generalised on the basis of the material itself, rather it is necessary to provide individual information on each of the certified elements as heterogeneity is property dependent.

2. Homogeneity statements cannot be given in absolute numbers but they are related to the mass consumed for analysis and can be given only on the basis of a certain statistical evidence.

3. In the case of biological RM's direct analytical methods using smaller sample intake than 10 mg for analysis are particularly suitable to assess the degree of homogeneity (heterogeneity). They do not need chemical sample pre-treatment and hence uncertainty contributions from digestion of samples does not influence the total variance of the repetitive measurements.

The statistical nature of the homogeneity problem can be treated theoretical and it is clear that it is a matter of large numbers. Either a large number of individual particles in the investigated unit or a large number of individual analysis will produce consistent results.

Therefore careful milling, grinding and mixing is essential in preparing sufficiently homogeneous RMs. The distribution of an element in a matrix is reflected in the repeatability of results obtained by an analytical technique of high precision and it is a function of the sample size used for analysis. The smaller the sample size, the lower the number of particles in a given analytical aliquot, the larger will be the spread in results of repetitive analysis. The mean value obtained from a number of investigations using 100 mg sample intake might be comparable to the mean value of a large number of results using only 1 mg samples, but the standard deviation calculated from the repetitive results will not be the same. Therefore the stated uncertainty in the certificates of RMs should be related to a specified mass for each element individually.

Practical aspects for the assessment of the degree of homogeneity in a sample can be summarised as follows:

Starting from a more qualitative point of view the particle size distribution of a material can give some indication of heterogeneity. The larger the number of individual particles in a certain mass aliquot is, the higher will be the probability to determine equal concentrations of an analyte in subsequent aliquots. Microscopic imaging can give additional information on the uniformity of the material.

Quantitative estimation of the degree of homogeneity is preferentially carried out by repetitive determination of analytes in the solid by a technique of known intrinsic precision. The total variance of the observations (analytical results), R^2_o is composed of the variance of the analytical method, R^2_a and the sampling variance from the heterogeneity of the study material, R^2_s.

$$R^2_o = R^2_a + R^2_s \qquad (1)$$

In order to extract the degree of homogeneity from the variance of repetitive determinations it is mandatory to determine the variance of the method used for analysis as accurately as possible.

The determined sampling variance of the material at a certain mass and the number of repetitive analysis can be used for the calculation of a sampling constant, k_s, a homogeneity factor H_E or a statistical tolerance interval ($m \pm \Delta$) which will cover at least a 95% probability at a probability level of $1 - \alpha = 0.95$ to obtain the expected result in the certified range [2].

The value of Δ is computed as a multiple of Rs, the standard deviation of the homogeneity determination, as $\Delta = k'_2 Rs$. The value of k'_2 depends on the number of measurements n, the proportion, P, of the total population to be covered (95%) and the probability level $1-\alpha$ (0.95). These factors for two-sided tolerance limits for normal distribution k'_2 can be found in various statistical text books [3].

Several practical approaches to checking homogeneity with the use of chemical analysis procedures are possible.

1. If RM is already distributed into individual bottles (units) one can check homogeneity by determining with the aid of INAA the content of several elements in several subsamples taken from one bottle and comparing them with analogous results in subsamples taken from various bottles chosen at random.

Comparing the variances of the two series of determinations by Fisher's F-test and the means by t-test at a significance level of e.g. 0.05 one can infer whether the two series of determinations differ significantly or not. If they do not, there is no justification to state that the material is inhomogeneous.

Care must be taken to limit as much as possible in such study the analytical variance. It is advisable to minimise all components of analytical variance. In INAA this would imply preserving good counting statistics (appropriately long counting times), estimation of neutron flux variability and assuring reproducible counting geometry etc.

2. Quantitatively the homogeneity (or inhomogeneity) for individual elements can be expressed with the aid of so-called Ingamells' sampling constants.

The overall relative standard deviation (in percent): $R_o = (s/\bar{x})\cdot 100$ as determined from a series of replicate samples of approximately equal masses is composed of analytical error R_a and an error due to sample inhomogeneity R_s.

As the variances are additive one can write:

$$R_s^2 = R_o^2 - R_a^2 \qquad (2)$$

Ingamells [4] introduced into analytical vocabulary the term "sampling constant" K_s defined as:

$$K_s = R_s^2 \cdot m \qquad (3)$$

where: R_s^2 is sampling variance and m is sample mass.

K_s is expressed in the units of mass and is numerically equal to the sample mass necessary to limit the error due to sample inhomogeneity (sampling uncertainty) to 1% (with 68% confidence).

Some workers have been using also $K_s^{1/2}$ for characterizing homogeneity of materials [5,6].

In order to determine sampling variance accurately, it is necessary to minimise as much as possible the individual components of analytical variance.

Once the sampling constant(s) are determined, one can predict what should be the magnitude of sampling variance for a given analyte and for various masses of the sample of a given material.

1.6 Experimental

1.6.1 *Neutron activation analysis (NAA) as a tool for checking homogeneity of reference materials*

NAA, because of its intrinsic features such as virtual absence of blank, good detection limits with respect to many elements, multielement capability, good penetration of neutrons through matter, small absorption of gamma rays in the analysed sample and good knowledge of potential sources of error, is well suited for checking homogeneity of relatively small masses of solid (powdered) natural matrix RMs. In addition, NAA offers a possibility of realistic estimation of the analytical error what, in combination with total variance of the determination, enables extracting the sampling variance.

$$R_s = \sqrt{R_o^2 - R_a^2} \tag{4}$$

Analytical variance of NAA is in turn composed of several components and in our case these are: counting statistics R_c, neutron flux inhomogeneity $R_{fi,}$ irreproducibility of counting geometry $R_{g,}$ and weighing R_w. So, if the separate components of analytical variance will be determined, the R_a can be obtained from the relation:

$$R_a^2 = R_c^2 + R_{fi}^2 + R_g^2 + R_w^2 \tag{5}$$

and then the sampling variance, R_s^2 and the error due to sample inhomogeneity R_s, can be derived from eq. (1). and (1a) respectively.

The results of activation analysis measurements are subject to well-known common analytical sources of uncertainties as well as method specific uncertainties. For NAA experiments intended to measure differences in induced activity, i.e. differences due to inhomogeneity in the amount of analyte in a given test portion, the experimental procedure can be designed to allow only the following uncertainties to be part of the result:

- uncertainty due to inhomogeneity R_s,
- uncertainty due to counting statistics R_c,
- uncertainty due to activation $R_{irr,}$ composed of
- uncertainty due to neutron fluence gradients,
- uncertainty due to changes in neutron energy spectrum,
- uncertainty due to irradiation time,
- uncertainty due to the gamma spectrometric measurement R_m, composed of
- uncertainty due to dead time,
- uncertainty due to pileup,
- uncertainty due to detector efficiency and resolution,
- uncertainty due to peak area determination,
- uncertainty due to radioactive decay correction.

Uncertainties relating to the determination of accurate quantitative results are not relevant in the context of determining homogeneity.

The determination and control of uncertainty due to counting statistics (R_c) is rather straightforward; this uncertainty is largely dependent on the sample composition, the decay characteristics of the indicator nuclides, and the assay parameters. An applied procedure can optimize irradiation, decay and counting parameters to obtain statistical uncertainties in the range of 1% to 0.1% for the analytes assayed. This requires peak areas of tens to hundreds of thousand counts; in the

case of rapidly decaying activities this can essentially be achieved with high count rate capabilities of the gamma spectrometers.

1.6.2 X ray emission techniques

Emission of the characteristic X rays of atoms excited by ionizing radiation is the basis of different X ray emission analytical techniques. The most important varieties are XRF (X ray Fluorescence), PIXE (Particle Induced X ray Emission) and EPXMA (Electron Probe X ray Microanalysis).

Thickness of the surface layer that contribute to the X ray yield is changing depending on the nature of the excitation radiation (photons, electrons, protons and heavier ions), energy of emitted X ray and sample matrix composition. Due to absorption, the highest contribution to the X ray yield comes from the sample surface. Depending on the source of excitation radiation, the area of the sample being exposed by the excitation beam can also vary significantly. Larger sample areas (mm^2 or cm^2 order) are irradiated if the broad beam of photons (from radioisotope source, X ray tube), or protons (from accelerator) is used. Much smaller dimensions can be irradiated by a probe techniques employing focused beams down to μm^2 (protons and X rays) or nm^2 (electrons) levels. It is clear that by the particular choice of X ray emission technique, sample portions being analyzed can be varied over many orders of magnitude.

Calculations of the sample mass being analyzed by particular method have to be carried out for every particular X ray energy (due to self-absorption), range of the excitation radiation and area of the sample being irradiated. Results of such calculations show that sample masses analyzed by X ray emission methods are typically in 0.1 -10 mg for broad beam methods (XRF and PIXE), while for the probe techniques (SRXRF, μPIXE, EPXMA) masses go down to μg and ng levels (see Table). Such small sample masses push requirements on the RMs homogeneity below the present availability.

In order to test the elemental homogeneity at these low sampling mass levels, X ray emission techniques offer numerous opportunities. In order to be able to consider these techniques for the homogeneity tests, it is essential to recognize the sources of uncertainties. The

overall variance R_o as determined experimentally from a series of measurements consists of the variance due to sample inhomogeneity R_s and the analytical variance R_a. Analytical uncertainties in X ray emission techniques can be grouped in three contributions:

- counting statistics including the spectrum fit errors (R_c);
- total exposure by incoming radiation including the dead time (R_N)- factor that is independent on the analyte and the sample;
- quantification procedure uncertainty R_Q — consisting from uncertainties in calculations or calibrations of detection efficiency (for particular X ray energy) and X ray yield (for particular geometry, sample matrix and X ray line).

with a final overall variance described as:

$$R_a^2 = R_c^2 + R_N^2 + R_Q^2 \qquad (6)$$

In attempt to improve ability to characterize the sample homogeneity, analytical uncertainty was minimized in some experiments by comparison of X ray intensities only (R_Q is neglected), or by suitable normalization (R_N is neglected).

Within the CRP, several approaches to characterize the homogeneity of the candidate reference materials by X ray emission methods were presented:

- Heterogeneity of the sample was studied by analysis of large number of single particles, using the computer controlled EPXMA.
- In the nuclear microprobe PIXE experiments scanning region was divided into the regions that are afterwards treated as the subsamples.

Independent X ray spectra obtained on different subsamples were normalized in the same way to minimize contribution of instabilities in intensity of the exciting radiation or its measurement:
- Ar X ray peak in SRXRF of subsequent sample regions.
  - X ray peak of element that is expected to show the highest degree of homogeneity.
- Heterogeneity in the material can be directly visualized by the nuclear microprobe PIXE imaging.

Essential ability to distinguish between the analytical and sampling contribution to the uncertainty in determination of levels of homogeneity is shown in most of these approaches. All presented techniques showed their suitability to be used for homogeneity testing on very low degrees.

Analytical errors in quantitative analysis by X ray emission analytical techniques are to a certain extent increasing due to the errors added in the quantification procedure (R_Q and R_N). In the case of PIXE and in particular in the application of fundamental parameter quantification procedure, sources of errors due to the normalization (charge collection and dead time) and thick target correction (uncertainty in matrix composition) are becoming more important. In such cases reference materials homogeneous on sample masses below 1 mg will play a key role in the further improvements in the accuracy and precision of these methods.

1.6.3 Solid sampling graphite furnace atomic absorption spectrometry, SS-AAS

The principle of atomic absorption spectrometry is described in various textbooks on spectrometry, e.g. Welz, Atomabsorptionsspektrometrie, Überlingen, 1990.

Two different approaches for background compensation exist — (i) the Zeemann splitting of excitation lines achieved by high magnetic fields either at the excitation lamp or at the place of atomisation, the graphite furnace, and (ii) D_2 compensation in parallel-beam instruments, where, by the help of a number of optical components, the continuous spectrum of D_2 light and the specific wavelength of the excitation lamp are chopped and guided through the cloud of atomised analyte. The resonance absorption of light by the analyte atoms in the graphite furnace leads to a negative signal which is converted into a peak area proportional to the number of excited atoms in the cloud.

Normally liquid samples are used in AAS determinations. The method, however, has such a high sensitivity for many elements that direct, solid sampling analysis is possible. In this variety of graphite furnace AAS small sample masses of the study material (from ~0.05 mg to ~2 mg for biological samples and ~50 mg for geological samples) are being weighed in small graphite boats using a micro-balance with accuracy of <1 μg and inserted manually into the graphite furnace. The graphite tube is transversally heated according to a temperature programme in order to (i) dry the sample, (ii) ash the sample and (iii) atomise the analyte element. Subsequently the furnace is heated for some seconds in excess to the atomisation temperature for burn out of possible residuals. Such a temperature cycle lasts for about 2 min. so that an experienced technician can handle 20 to 30 analyses per hour [7].

Quantification is carried out by calibration of the instrument either with liquid standards or with solid standards (certified reference materials). As atomic absorption spectrometry to a certain extent is matrix dependent, the choice of the RM for calibration is critical. The linear range of the technique is for most elements limited to about one order of magnitude and therefore a reference material of similar matrix composition and concentration in the relevant range compared to the study material should be used. In many cases one has to compromise these requirements as no directly comparable RMs might be available. On the other hand reference materials available today are

certified on the base of at least 100 mg sample mass and no particular information on the homogeneity of individual elements is provided in the certificate. Therefore exact quantification by SS-AAS seems to be difficult, at least at the present situation of resources for calibration.

The precision of the method is, however, good and therefore SS-AAS is very much suited to determine the degree of heterogeneity of single elements in natural materials.

The analytical variance R^2_S is composed of several components such as intensity fluctuations of the excitation lamp R^2_I, the uncertainty of the temperature generated during atomisation R^2_A, chemical interactions in the vapour phase of the analyte with other excited atoms from the matrix R^2_C, electronic noise in the processing of the signal intensity R^2_N and the variance from weighing uncertainty R^2_W.

$$R_S = \sqrt{R^2_I + R^2_A + R^2_C + R^2_W} \quad (7)$$

As an example the variance of the measurement process has been experimentally estimated by repetitive measurements of a liquid standard of 0.1 ng/ml Pb. The total standard deviation of 40 repetitive analysis of 5 ml portions was 3.2%. Assuming a pipetting error of ≤1% (determined by weighing) a total uncertainty of the measurement process of 2.2% for Pb in the low ng/g range remains. These uncertainties have to be evaluated for each of the elements separately.

1.7 Preparation of candidate CRMs

1.7.1 General

For reference materials for microanalytical techniques special precautions and requirements are necessary not only for sample collection but also for sample preparation. Some of the existing techniques were tested and modified as part of the CRP. Special precautions need to be made to avoid collection of other matrices as part of the sample (e.g. soil or dust particles together with biological samples, bark together with the lichen, blood tubes together with the liver). These would complicate the homogenisation of the material and could lead to less suitable materials. In addition every contamination is more likely to influence the quality of this type of RMs and need to be avoided. Therefore it is very important to document all details of sample collection and sample preparation equipment to trace back eventually determined contamination and inhomogeneities (see also the comprehensive treatment of this subject in: Stoeppler, M., Wolf, R.W., Jenks, J., (eds.) Reference Materials for Chemical Analysis, Wiley-VCH, Weinheim, (2001)).

1.7.2 Sample preparation

Reference materials for microanalytical techniques require special precautions not only for sample collection but also for sample preparation. Some of the existing techniques were tested and modified as part of the CRP. Special care to avoid contamination of samples with particles of other matrix types during sampling should be applied (e.g. soil or dust particles together with biological samples, bark together with lichens, blood tubes together with liver, etc.). These would complicate the homogenization of the material and could lead to less suitable materials. In addition every external contamination is definitely jeopardizing the quality of this type of RMs and need to be carefully avoided. It is very important to document all details of sample collection and sample preparation, the used equipment and personnel to trace back eventually encountered contamination and heterogeneities.

Due to their fine particle size naturally homogeneous materials such as single cell algae or deep-sea sediment showed no need for sample preparation except spray drying. Their natural particle size distribution is comparable to particle sizes normally achieved after several milling steps (see

example of Algae IAEA393 in figure 1). The usually applied drying procedures such as lyophilisation were not applicable because they produced a 'concrete like' block, which could not be brought to the powdered form again without milling. This increased the risk of contamination and destroyed the original structure (e.g. cell structure of algae) of these materials. To maintain the fine particle size and structure of naturally grown or formed material with naturally small particle size drying with a spray dryer was the method of choice.

For all other samples, the reduction of sample particle size was the most important sample preparation and was composed of two steps, the initial grinding and the fine milling. Two differed milling procedures were tested during the CRP: the jet milling (for IAEA-338, IAEA-395) and the use of ball milling (IAEA-386). Both methods were suitable, but needed to be applied more than once to produce the desired low particle sizes. Figures 1 and 2 show the improvement of the particle size distribution of Lichen IAEA-338/pretest and Urban Dust IAEA-396/A. The link between improvement in particle size and improvement of homogeneity is shown in table 2. Sampling constant (the mass which may be used to get 1% of sampling error at 65% confident limit) improved from a factor of 1.2 for Sc up to a factor of 800 for Au. The average improvement was about a factor of 2–10.

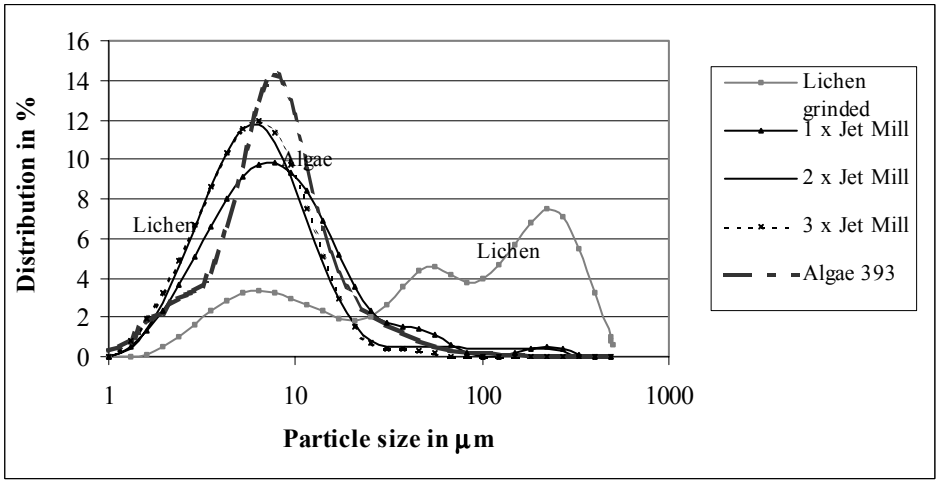

Figure 1. Particle Size Distribution of Algae and Lichen Candidate Reference Materials.

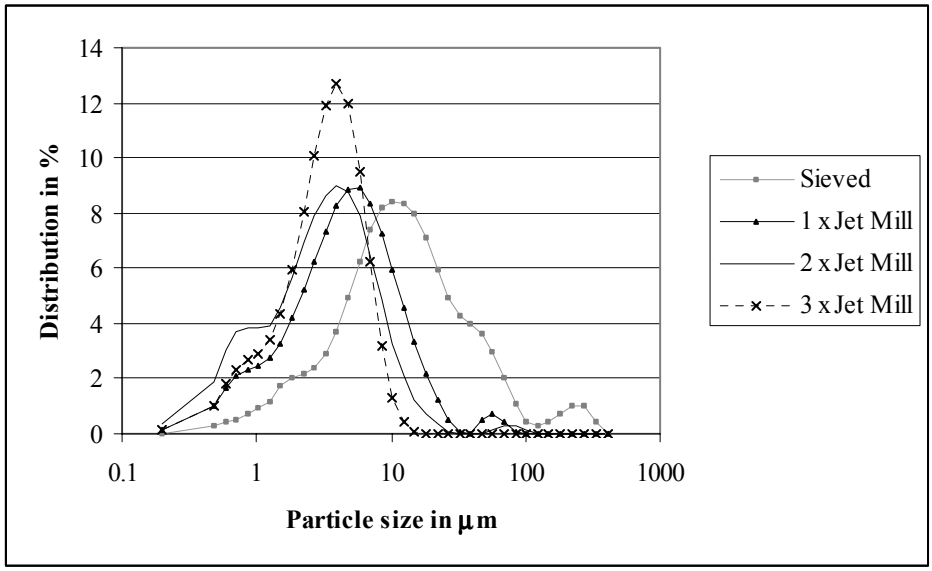

Figure 2. Particle Size Distribution of Urban Dust Candidate Reference Material.

1.8 Analytical results

The participating laboratories analysed several new candidate CRMs that were identified and/or developed for the use with microanalytical techniques. In addition, a number of already existing CRMs have been investigated. The detailed data are reported in the participants' working data. A major effort has been spent on the characterisation of two IAEA materials that had been specifically designated for investigation in this CRP; these are IAEA-338, Lichen, and IAEA-413, Algae (Elevated Level). The summary of the data is shown in Tables II and III. The tables list the methods used, the sample sizes and number of samples investigated and presents the analytical results that are relevant to the evaluation of the elemental homogeneity of the materials: the sample mass used for analyses, R_0, R_A, R_S and Ingamel's sampling constant K_S.

As the different techniques applied to the materials used very different sample mass, from 0.0001 mg to 120 mg, it was questioned if the theory could cover these 5 to 6 orders of magnitude and a strictly linear relationship of K_S with the mass can be assumed. The evaluation of the data from Tables II and III showed that this assumption can be readily made and additionally the plots prepared from the data could be used for the identification of outlying results. In Figures 1 to 10 K_S results for elements with more than 4 data points from IAEA 338 are plotted versus the mass analysed. In Figures 11 to 20 similar plots are displayed for IAEA 413. For better resolution of the single data points the mass axes had to be converted into log10. The slope of the relationship between K_S and m as given in Table IV is equal to R_S^2 — the variance due to the elements distribution — and is directly reflecting the level of homogeneity of the particular element in the specific material.

Table II: Comparison of K_S factors of sieved and jet milled IAEA- 396/A Urban Dust

Element	*K_S Factors*	
	IAEA 396/A sieved	*IAEA 396/A jet milled (3x)*
As	45	30
Au	13204	166
Ba	24	5
Br	1	7
Ca	62	18
Ce	108	7
Co	12	7
Cr	110	0.4
Cs	3	21
Eu	139	69
Fe	0.2	0.1
Hf	822	51
La	127	6
Lu	308	83
Mo	123	83
Na	6	2
Rb	31	15
Sb	3	1
Sc	5	4
Sm	106	21
Ta	35	35
Th	107	4
Zn	6	12

TABLE II. variance of element distribution R_S^2 in IAEA-338 (lichen) and IAEA 413 (single cell algea, elevated level). Missing values due to insufficient number of analytical techniques applied (< 4 techniques)

Element	IAEA-338	IAEA-413
Cl	6.948	
K	8.876	5.024
Ca	7.258	13.054
Cr	2.085	2.521
Mn	1.86	4.426
Fe	2.64	2.588
Zn	2.579	3.874
As		1.306
Br	8.508	7.43
Rb	2.118	
Cd		1.726
Hg		293.
Pb	95.82	

As one can see from the Table each element exhibits its own characteristics in the particular material. The transition metals together with As and Cd seem to be more homogeneously distributed in both materials compared to K, Ca, Cl and Br. Obviously Hg in the 413 material and Pb in the 338 material are very badly distributed. Relatively large minimal sample mass would result for the determination of these two elements at a given confidence level to a defined repeatability. As these R_S^2 values have been determined by a wide variety of independent techniques over a large range of sample mass these values could well be used to determine uncertainties of other techniques using the approach of repeatability measurements as outlined in this TECDOC.

Using a criteria of $R_S^2 \times 10$ suggested to be sufficiently homogeneous (K_S for 1 mg sample mass = 10), the evaluation gave satisfactory results for a number minor and trace elements in IAEA-338: Cl, K, Ca, Cr, Mn, Fe, Zn, Br, and Rb. Other trace elements of environmental or biological significance also showed good homogeneity: Na, Al, S, Mg, V, Co, Cu, Sc and Sb. In all instances the findings were confirmed with reasonably low uncertainties obtained by techniques that use variable sample sizes. Since the major use of this future CRM will likely be in the area of environmental biomonitoring, additional investigations should be conducted to possibly include critical elements such as Ni, Se and Hg into the investigation. It is recognised that some of the less certain results on these elements may be due to insufficient detection sensitivity of the currently applied techniques. Also elements that are susceptible to external contamination during preparation and analysis, e.g. Si and Co need additional confirmation prior to a conclusive deposition.

For IAEA-413, the evaluation gave satisfactory results for a significant number of minor and trace elements: Na, S, K, Ca, Mn, Fe, Co, Zn, As, and Br. The elements that were artificially elevated during the cultivation of the algae to simulate highly polluted environmental situations, As, Cd, Cr, but not Hg, demonstrated homogeneity for the investigation with microanalytical techniques. For two other pollutant elements that had been added during the cultivation, Ni and Pb the investigations did not produce conclusive data. This should be confirmed with additional measurements at the milligram level. The data tend to indicate that microprobe techniques may be confronted with heterogeneity at

very small sample size levels that are due to different composition of small units of the sample, i.e. particles of different morphology or origin than the bulk of the matrix.

1.9 Conclusions

It was confirmed by the results obtained through this CRP that element specific sampling uncertainties can be obtained that meet the criteria for homogeneous distribution of elements in existing CRMs as well as in candidate CRMs as defined by the RCM members. The experiments carried out by the participants on low sample mass analysis brought up a number of highlights:

- It was recognized that Ingamels' sampling constant as well as related concepts are appropriate models to describe the sampling behavior of elements in well mixed solid materials at sample sizes between 0.0001 mg and 120 mg.

- Analytical techniques suitable for homogeneity testing should meet certain criteria such as i) sample weight for analysis should be less than 10. mg, ii) preferentially it should be a direct method using solid material for analysis, iii) results should be obtained from more than 10 independent aliquots of the same material, vi) the analytical uncertainty should be well understood and sufficiently small to detect sampling uncertainty ($R^2_S/R^2_A > 1$, or $R_O \leq 2.5\%$). It was found that nuclear techniques such as INAA and μ-PIXE meet these criteria.

- Preparation of Reference Materials is critical with respect to the final particle size distribution, which should exhibit low maximum (≤ 50 μm) and a narrow range. Milling techniques to meet such criteria are available today. Materials that show intrinsic uniformity are particularly suitable.

- In the following CRMs some elements have been identified to show homogeneous distribution (according to the criteria given above) on a 1 mg sample mass within a 5% probability at a 95% confidence level:
  IAEA-338, Lichen
  IAEA-413, Algae

Additionally other existing CRMs such as CTA-VTL2 (Virginia tobacco), IAEA-SD-N-2/TM (lake sediment), GSPN-2 and -3 (manganese noodles), SRM 1547 (peach leaves) and SRM 1649 (urban dust) have been assessed. Further matrices such as deep sea sediment, air particulate and bovine liver are under investigation.

By presenting precise information on the element specific sampling uncertainties in reference materials users of CRMs will obtain a valuable tool for:

calibration of microanalytical techniques using much smaller sample mass than has been recommended in previous certificates of CRMs, and
estimation of the individual method derived uncertainty by comparison of the total uncertainty with the given sampling uncertainty for the investigated element.

This will open a new kind of use for CRMs as total uncertainty budget determinations are essentially required to establish traceability for micro-analytical techniques. By supporting this CRP, the IAEA has contributed to a significant expansion of technical capabilities in several Member States' laboratories. This new technology can be widely used in the Member States to improve national and regional reference materials programs. The participants in this RCM envision that future research would refine and expand the knowledge gained thus far to include samples of the nanogram range in the measurements and models describing homogeneity and to further evaluate, minimize, and accurately quantify the analytical uncertainties of nuclear and related analytical techniques. This ultimately will significantly expand the utility of such techniques in certification and quality control, as well as in field applications.

2. MAIN IMPACT OF THE CRP

For more than 30 years the Analytical Quality Control Services (AQCS) programme of the IAEA's laboratories has been supporting its member states through the preparation and distribution of reference materials (RMs). These RMs fulfil an important role as quality control materials which are needed by MS laboratories to demonstrate the performance of their analytical techniques and to assure the quality of their results. Most of the AQCS customers work in nuclear fields and/or are using nuclear or nuclear related analytical techniques which include: Instrumental Neutron Activation Analysis, Accelerator Mass Spectrometry, Proton Induced X ray Emission (PIXE) and micro-PIXE analysis, and many X ray based techniques. Many of these nuclear and nuclear related techniques are capable of performing analysis on very small samples (ranging in mass from mg down to μg) where the sample size may be limited due to sample availability (tissue sample) or sample cost (rare artefacts). Unfortunately no RMs were available to meet the needs of the scientific community who employ microanalytical nuclear techniques and this deficiency has led to a lack of evidence regarding the quality of results which in turn affected the credibility of the results from these techniques. By preparing RMs for these types of studies, the IAEA intended to provide tools to the MS to enable them to demonstrate the applicability of nuclear and isotopic techniques in human nutrition research, in studies of non-radioactive environmental pollutants and to prove their analytical reliability.
During this CRP developments were made, technically and theoretically to set the basis for the preparation and characterisation of RMs needed for these analytical techniques:

- Significant improvements were made in the measurement precision of the various nuclear and nuclear related techniques necessary to determine the homogeneity for these very low concentrations of a number of elements in sample sizes ranging from picograms to 10 mg.
- The CRP was successful in confirming that two candidate IAEA Reference Materials were suitable for quality control in nuclear microanalytical techniques. A worldwide intercomparison exercise and a proficiency test is planned for the year 2000.
- Guidelines were developed for use by other laboratories involved in the preparation and characterisation of new RMs of this type.
- It was confirmed that Ingamel's sampling constant, as well as related concepts were appropriate models to describe the sampling behaviour in homogenous samples used as RMs for nuclear and nuclear related microanalytical techniques.

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INDIVIDUAL CONTRIBUTIONS

Assessment of homogeneity of candidate reference material at the nanogram level and investigation on representativeness of single particle analysis using electron probe X ray microanalysis

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Abstract. Particulate samples of a candidate reference material are evaluated on their homogeneity from bottle to bottle using electron probe X ray microanalysis technique. The evaluation on the homogeneity is done by the utilization of the Kolmogorov — Smirnov statistics to the processing of the quantitative electron probe X ray microanalysis data. Due to a limitation, existing even in computer controlled electron probe X ray microanalysis, in terms of analysis time and expenses, the number of particles analyzed is much smaller compared to that in the sample. Therefore, it is investigated whether this technique provides representative analysis results for the characteristics of the sample, even though a very small portion of the sample is really analyzed. Furthermore, the required number of particles for the analysis, to insure a certain level of reproducibility, e.g. 5% relative standard deviation, is determined by the application of the Ingamells sampling theory.

Keywords

reference materials, electron probe X ray microanalysis, homogeneity, Kolmogorov — Smirnov statistics, Ingamells sampling theory

Introduction

Homogeneity is one of the essential attributes of reference materials. Normally its estimation is a two-step process. In the first step, one or a number of bulk analytical techniques (most often neutron activation analysis (NAA), atomic absorption and emission, X ray fluorescent (XRF) and/or mass spectrometry [1]) are used. The statistical evaluation of the obtained data is done in the second step (for details see e.g. reference [2]). Still, such approach allows to evaluate the homogeneity of the reference materials (RMs) only down to the microgram level and cannot be applied to lower amounts [3].

In order to reach the nanogram level, one should use microanalytical rather than bulk techniques. However, the assessment of homogeneity of the samples of candidate RMs with the help of microanalytical techniques cannot be considered as a routine procedure due e.g. to the fact that the majority of microanalytical techniques are not standardized themselves. Electron probe X ray microanalysis (EPXMA), which is widely used for qualitative and quantitative analysis of individual particles, is not an exception. On the other hand, computer controlled EPXMA (CC EPXMA) is capable to determine the compositions of large number of individual particles in an automatic and rather non-destructive manner. Therefore, its application to homogeneity studies of powder samples looks very attractive.

The present paper describes an approach, which allows to apply EPXMA to the estimation of the homogeneity of the powder samples of candidate RMs. It is based on the utilization of the Kolmogorov — Smirnov statistics to the processing of EPXMA data.

Single particle analysis using CC EPXMA is an ultimate microanalysis technique available currently; it analyzes individual particles of micrometer size. Morphological and chemical information on individual particles can be obtained. Since EPXMA analysis time, and thus analysis expenses, increase by the increase of the number of analyzed particles, the number of particles analyzed are strictly limited, even with CC EPXMA. And thus, it is important to know whether the information obtained from a very small portion of sample represents the characteristics of sample. One of objectives of this work is to investigate how a small portion of the sample can be analyzed to insure representative analysis on the sample.

Experimental

Samples and sample preparation.

A candidate RM, namely IAEA-413, which is single cell algae grown with added toxic elements, is studied in the current research. This candidate RM is produced by re-mixing a candidate RM which was previously characterized using various analytical techniques, such as NAA, XRF, particle induced X ray emission (PIXE) and EPXMA [3,4] Six different bottles out of an RM batch were sampled. Since the samples were dry powders, they could not be analyzed directly by EPXMA but had to be transferred and spread onto Nuclepore polycarbonate filter. The Nuclepore filter is an ideal substrate for the CC EPXMA because of its microscopic flatness. Well separated particles were produced by the liquid suspension technique as follows [5]. A small portion of the powder is dispersed in an inert liquid, *n*-hexane. From this suspension the appropriate amount, to get an optimal loading, is pipetted into a filtering funnel with vertical sides, filled with *n*-hexane. This suspension is then sucked through a 25 mm Nuclepore filter, 0.4 μm pore-size, supported by a glass filter. Six samples from the different bottles of the candidate RM were analyzed. A sample from a bottle was repeatedly analyzed to investigate the reproducibility of the obtained data using CC EPXMA.

CC EPXMA

Analysis of the samples was done on these filters, after carbon coating to avoid charging, using a JEOL 733 scanning electron microscope (SEM) with energy-dispersive X ray (EDX) detection attachment. Automated single particle analysis was performed and 2,000 particles were analyzed for each sample. For the analysis an accelerating voltage of 20 kV and a beam current of ca. 1 nA were used. X ray spectra, collected for 20 seconds, provided information about the chemical composition of the individual particles, and also morphological information, such as diameters, was determined. The magnification of 300, used in the measurements, determined the minimum detectable diameter, which is about 1 μm.

Results and discussion

Summary on procedures for data analysis

- **Evaluation of the total mass of the analyzed microparticles per sample.**

A very rough evaluation of the total mass *M* of the *N* analyzed microparticles per sample was done as follows. Let an average microparticle have an average volume V and average density p. Then

$$M = N * p * \nu \qquad (1)$$

The average density is roughly estimated as 0.7 g/cm^3 which is the density of *n*-hexane (some of the particles in suspension with *n*-hexane had a density lower than that of *n*-hexane, some higher). The average volume can roughly be estimated from the size distributions of the particles assuming that they are spherically shaped (according to SEM observations). Based on these data we estimate the average volume as 220 μm^3. Hence, the total mass, *M,* is estimated as 300 ng when 2,000 particles are analyzed.

- **Assessment of the reproducibility of sampling**

To estimate the reproducibility of sampling, the size distribution (distribution of the diameters) of the particles for the six samples was determined. The size distribution of each sample was compared with one of the samples (the target sample), arbitrarily chosen, with the help of the two-sided Smirnov statistical test.

The Smirnov statistical test is to determine whether two distributions of data are identical or not. This test belongs to the variety of nonparametric statistical tests. Other tests, e.g. the *t* test, can be used too, but the advantage of this Smirnov test is its consistency against all types of differences that may exist between two distributions. To the contrary, the *t* test assumes that the distributions to be tested are normal distributions. For more detail on this test, one can refer to the book by Conover [6] and also to a work [4] where a similar candidate RM was investigated using the Smirnov test.

This test is applied in the following way:

(a) Each distribution is normalized on a maximum value. Therefore, after normalization, the maximum value in each distribution equals 1.

(b) The difference between the target distribution and that for a sample (Δ) is calculated for each bin, where the number of bins is 50.

(c) The maximum value of Δ's is compared with a certain critical value *T* for a certain significance level taken from the two-sided Smirnov test tables [6]. If the maximum value of Δ's > *T,* the difference between the distributions is considered significant; otherwise it can be neglected.

- **Evaluation of the composition differences between the samples from different bottles**

To evaluate composition differences between the samples, the distributions of concentrations of the six most often detected elements were investigated for each sample. These concentration distributions for each sample were compared then with the target one with the help of the two-sided Smirnov statistical test as described previously. Also, for each measured data with total 2,000 particles, data with a smaller number of particles, such as 1800, 1500, 1200, 900, 600, 300, 100 and 50, were generated by selecting particles randomly from the original data, using a random number generating function in the Microsoft Excel program. The data with the smaller number of particles were compared again to check whether their compositional distributions are identical also in the smaller mass range, using the Smirnov test.

- **Assessment on representativeness of the data measured using CC EPXMA**

One sample from a bottle was repeatedly analyzed seven times during a two months period and at different areas of the loaded filter, to investigate whether each data is reproducible. The reproducibility was checked using the Smirnov test. Also, it was evaluated how many particles need to be analyzed to achieve a certain relative standard deviation (RSD) for the measurement, e.o. a certain level of reproducibility. For this purpose, Ingamells sampling theory [7] was applied to determine what mass of the sample is needed to achieve a certain level of reproducibility. In the Ingamells theory, therequired sample weight, *w,* if the sample is homogeneous, to achieve 1% RSD at 68% confidence, is given in Eq. 2.

$$K_s = R^2 * \omega , \qquad (2)$$

where K_s is sampling constant which is the sample weight to achieve 1% RSD at the 68% confidence level and *R* is RSD in%. When K_s is obtained from measurements, required sample mass to achieve a certain level of reproducibility is easily calculated from Eq. 2.

Using the theory, it was determined how many particles need to be analyzed in CC EPXMA analysis, to ensure 5% RSD for each chemical element.

Results

- **Homogeneity test from bottle to bottle for the IAEA candidate RM**

In the previous study, the candidate RM was characterized using various analytical techniques [3,4]. Since the candidate RM was observed to have variations in its composition from bottle to bottle, the EPXMA technique was used to investigate which bottles are different from others, if there are, at the nanogram level, which is not possible to check with other analytical methods.

The size distributions of samples from different bottles were tested, whether they are same or not, on the basis of the two-sided Smirnov statistics. They were compared with a target sample from a bottle, namely "bottle 8", which is arbitrarily chosen out of six available bottles. As an example, the size distributions are shown in Fig. 1. The distributions are not normal distributions, so that the Smirnov test, which does not assume that the distributions of interest are normal distribution, is used in this work. The results of the statistics, namely the maximum difference between the target distribution and those of different samples along with corresponding critical values *T,* are given in Table I (the results of the first row in Table I is for the size distributions; the others for elemental concentrations.). It was found that, with a probability $p = 0.90$. the size distributions are identical for all the bottles except bottle 40. As shown in Fig. 1, the size distribution of bottle 40 is significantly different from others. Also, the average values of the particle diameters from different bottles are shown in Fig. 2 to demonstrate visually that the average diameter of bottle 40 is not same as for the others. Even if all the samples, except one of bottle 40, are same in their sizes at the 300 ng (equivalently, 2,000 particles) level, their homogeneity is not guaranteed at the lower mass range. The number of particles in each data was reduced systematically, using a random number generator of the Microsoft Excel software, from 2,000 and down to 50. In terms of the mass of the analyzed samples, the minimum mass investigated is about 8 ng (50 particles). As shown in Table II, they are the same down to about 15 ng (100 particles). At the 8 ng level, the sample from bottle 32 is different from the others. In other words, the sizes of samples from different bottles, except bottle 40, are the same down to 15 ng level.

TABLE I. RESULTS OF THE SMIRNOV TEST OF SAMPLES FROM DIFFERENT BOTTLES TO THE TARGET SAMPLE (SIGNIFICANT LEVEL $P = 0.90$ AND CRITICAL VALUE $T = 0.24$). THE NUMBER OF PARTICLES IN EACH DATA IS 2,000 (ABOUT 300 NG). DIFFERENT DISTRIBUTIONS FROM THE TARGET DISTRIBUTION ARE SET IN BOLDS.

Variable	Bottle 16	Bottle 24	Bottle 32	Bottle 40	Bottle 48
	Maximum difference between distributions				
Diameter	0.06	0.12	0.16	**0.39**	0.01
Mg	0.02	0.02	0.02	0.06	0.01
P	0.02	0.03	0.19	**0.62**	0.10
S	0.02	0.04	0.13	**0.54**	0.08
K	0.03	0.03	0.19	**0.59**	0.08
Ca	0.02	0.03	0.05	**0.35**	0.05
Fe	0.01	0.01	0.01	0.07	0.03

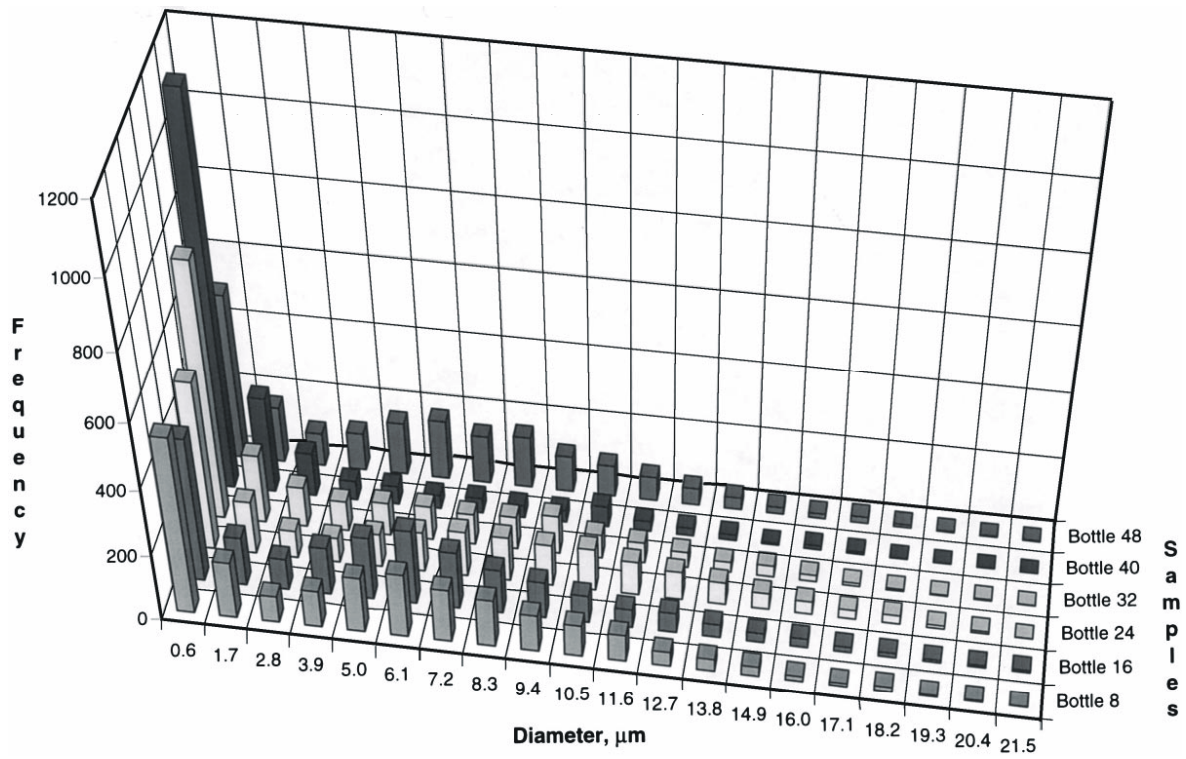

FIG. 1. *Size distributions for six samples from different bottles.*

TABLE II. RESULTS OF THE SMIRNOV TEST FOR SIZE DISTRIBUTIONS OF SAMPLES TO THE TARGET SAMPLE (SIGNIFICANT LEVEL P = 0.90 AND CRITICAL VALUE T = 0.24). THE NUMBER OF PARTICLES IN EACH DATA IS VARIED DOWN TO 50. DIFFERENT DISTRIBUTIONS FROM THE TARGET DISTRIBUTION ARE SET IN BOLDS.

No. of particles	Bottle 16	Bottle 24	Bottle 32	Bottle 40	Bottle 48
	Maximum difference between distributions				
2,000	0.06	0.12	0.16	**0.39**	0.01
1,800	0.06	0.12	0.16	**0.39**	0.02
1,500	0.06	0.12	0.17	**0.40**	0.02
1,200	0.07	0.12	0.18	**0.40**	0.02
900	0.07	0.14	0.15	**0.36**	0.03
600	0.10	0.14	0.15	**0.36**	0.07
300	0.04	0.17	0.15	**0.33**	0.05
100	0.10	0.10	0.17	**0.44**	0.16
50	0.12	0.14	**0.26**	**0.34**	0.20

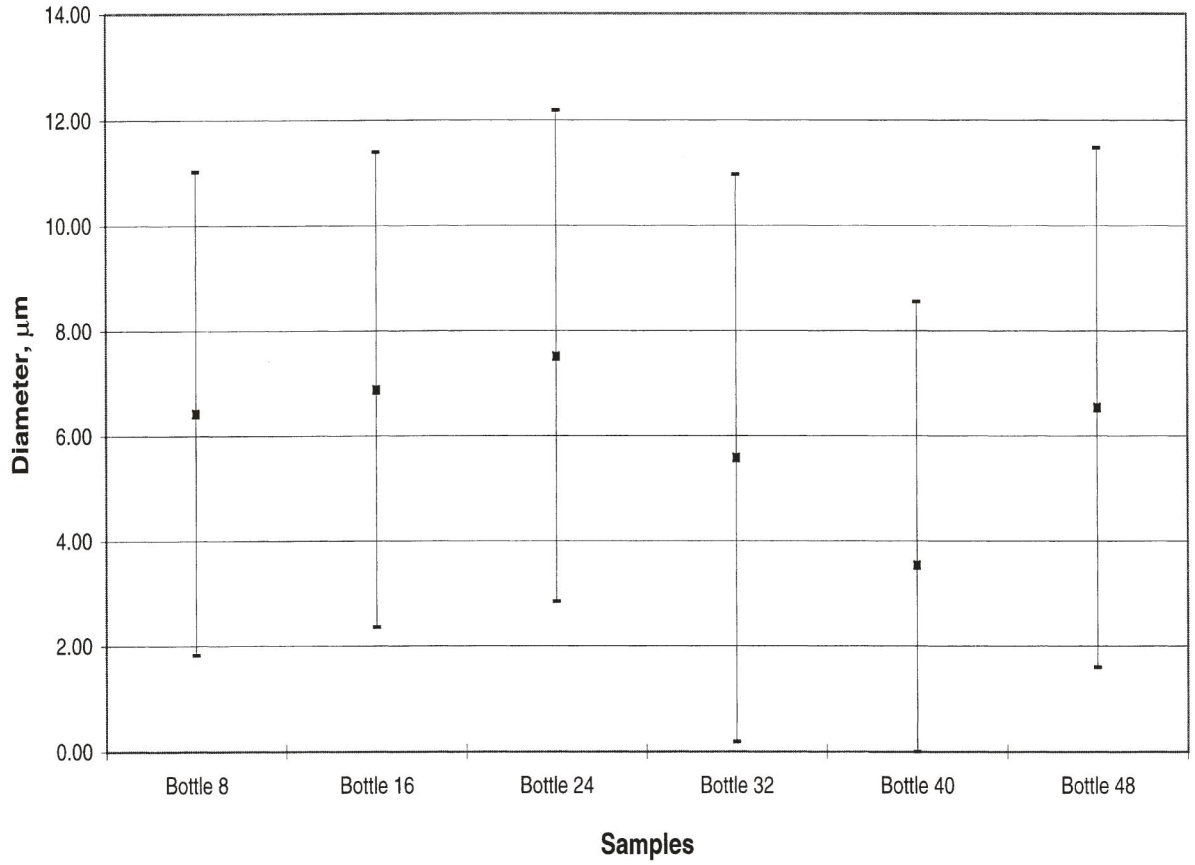

FIG. 2. *Averages of diameters, with their standard deviations, for six samples.*

For the candidate RM, Mg, P, 5, K, Ca, and Fe were the most often met elements. Among those elements, P, 5, and K are major elements. Their average concentrations, which were obtained from the intensities measured by Si(Li) EDX detector, range from 20% to 40% for all the analyzed samples. Mg, Ca, and Fe are minor elements, of which the concentrations are in the range from a percent to several percents. The similarity of the concentration distributions of these elements for each sample was tested, in the same way as it was done for the size distributions. The result of the two-sided Smirnov statistical test for the data of 2,000 particles is given in Table 1. They are all same, except bottle 40, with a probability as high as $p = 0.90$. In other words, all the samples, except bottle 40, are the same in their compositions at the 300 ng level. Bottle 40 is different for the major components such as P, S, and K, and also the minor components, such as Ca. The distributions of the Mg and Fe concentrations are all same including bottle 40; the reason for this is not clear, though. In Fig. 3, as an example, the distributions of the S concentrations are shown to demonstrate visually that bottle 40 is different from the others. Also, the number of particles in each data was reduced in the same way as it was done for the size distributions. One of the samples starts to be different from others at about 8 ng level. As shown in Table III, the sample from bottle 32 is different from the others for one of the major components, namely K. However, the difference also might be due to the instrumental instability; from the repeated measurements for a sample, it was found that the measurements do not provide reproducible results at this very low level, as described in the next section.

- **Reproducibility of repeated measurements for a sample prepared from a bottle**

A sample from bottle 8 was analyzed seven times repeatedly at different times and at different areas of the loaded filter; there are a huge number of particles on the loaded filter and only a very small part of the sample (2,000 particles each time) is analyzed using CC EPXMA.

TABLE III. RESULTS OF THE SMIRNOV TEST FOR COMPOSITIONAL DISTRIBUTIONS OF SAMPLES TO THE TARGET SAMPLE (SIGNIFICANT LEVEL p = 0.90 AND CRITICAL VALUE T = 0.24). THE NUMBER OF PARTICLES IN EACH DATA IS 50 (ABOUT 8 ng). DIFFERENT DISTRIBUTIONS FROM THE TARGET DISTRIBUTION ARE SET IN BOLDS.

Variable	Bottle 16	Bottle 24	Bottle 32	Bottle 40	Bottle 48
	Maximum difference between distributions				
Mg	0.14	0.08	0.06	0.02	0.04
P	0.10	0.20	0.16	**0.52**	0.12
S	0.12	0.08	0.16	**0.42**	0.16
K	0.14	0.10	**0.28**	**0.58**	0.12
Ca	0.06	0.02	0.06	**0.44**	0.10
Fe	0.02	0.04	0.06	0.04	0.04

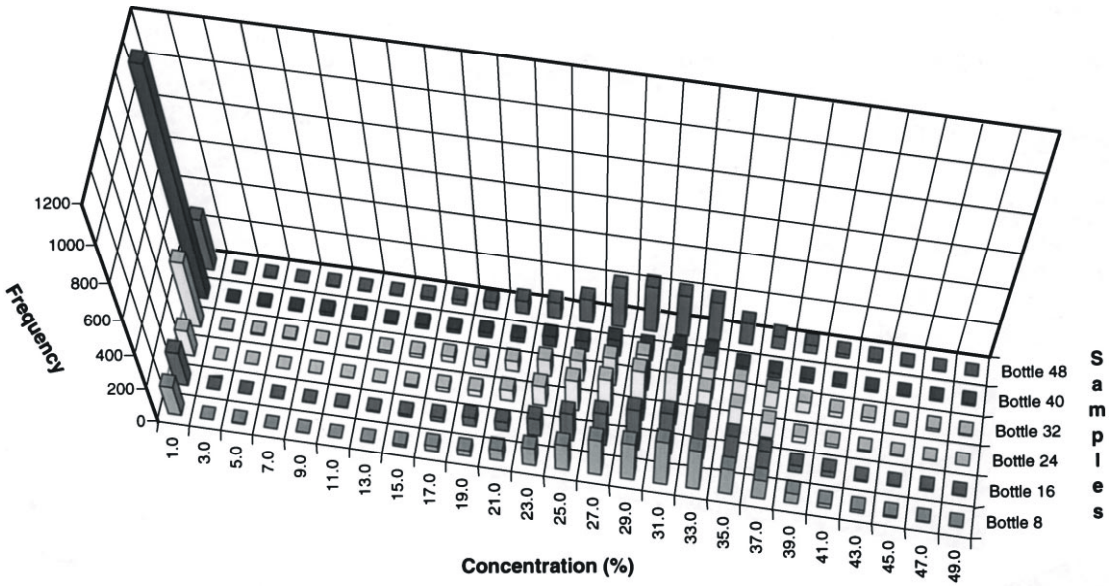

FIG. 3**.** *Distributions of S concentration for six samples.*

The similarities of the size distributions of those data were tested, on the basis of the two-sided Smirnov statistics. As an example, the size distributions and the results of the statistics along with corresponding critical values *T,* for the data of 2,000 particles each, are given in Fig. 4 and Table IV. It was found that, with a probability p = 0.90, the size distributions are identical for all the data. Even if all the data are the same in their sizes at the 300 ng level, their homogeneity was checked at the lower mass ranges. Also, the number of particles in each data was reduced in the same way as it was done previously. As shown in Table V, they start to be different at the 8 ng (50 particles) level. At the 8 ng level, two among seven data are different from the others. In other words, the sizes of the particles of a sample are the same for the different measurements at the 15 ng level.

Again, the similarity of the concentration distributions of the elements for each data was tested, in the same way as it was done for the size distributions. The result of the two-sided Smirnov statistical test for the data of 2,000 particles is given in Table IV. They are all the same with a probability as high as p = 0.90. In other words, all the different areas in the loaded filter, measured at

the different times, are the same for their compositions at the 300 ng level. Also, the data with a reduced number of particles were examined. As shown in Table 6, they start to be different at the 15 ng level. At the 15 ng level, two among seven data are different from the others for one of the major components, namely K. At the 8 ng level, four among seven data are different from the others in the major components, namely K and S. In other words, the data obtained from the same sample are reproducible for its compositions down to 45 ng (300 particles) for this candidate RM, regardless of what part of the sample, and also when it is measured. This result means that the single particle analysis using CC EPXMA provides representative information both for morphology and compositions on particulate sample, even though a very small portion of the sample is analyzed.

TABLE IV. RESULTS OF THE SMIRNOV TEST OF DATA FROM THE BOTTLE 8 TO THE FIRST DATA (SIGNIFICANT LEVEL P = 0.90 AND CRITICAL VALUE T= 0.24). THE NUMBER OF PARTICLES IN EACH DATA IS 2,000 (ABOUT 300 ng). ALL THE DISTRIBUTIONS ARE SAME, WITH NO MAXIMUM DIFFERENCE EXCEEDING THE CRITICAL VALUE.

Variable	Data 2	Data 3	Data 4	Data 5	Data 6	Data 7
	Maximum difference between distributions					
Diameter	0.08	0.16	0.19	0.19	0.16	0.09
Mg	0.02	0.01	0.01	0.02	0.01	0.01
P	0.05	0.05	0.04	0.08	0.05	0.05
S	0.09	0.15	0.12	0.14	0.15	0.06
K	0.14	0.18	0.15	0.13	0.18	0.08
Ca	0.01	0.02	0.01	0.03	0.02	0.01
Fe	0.01	0.01	0.01	0.01	0.01	0.00

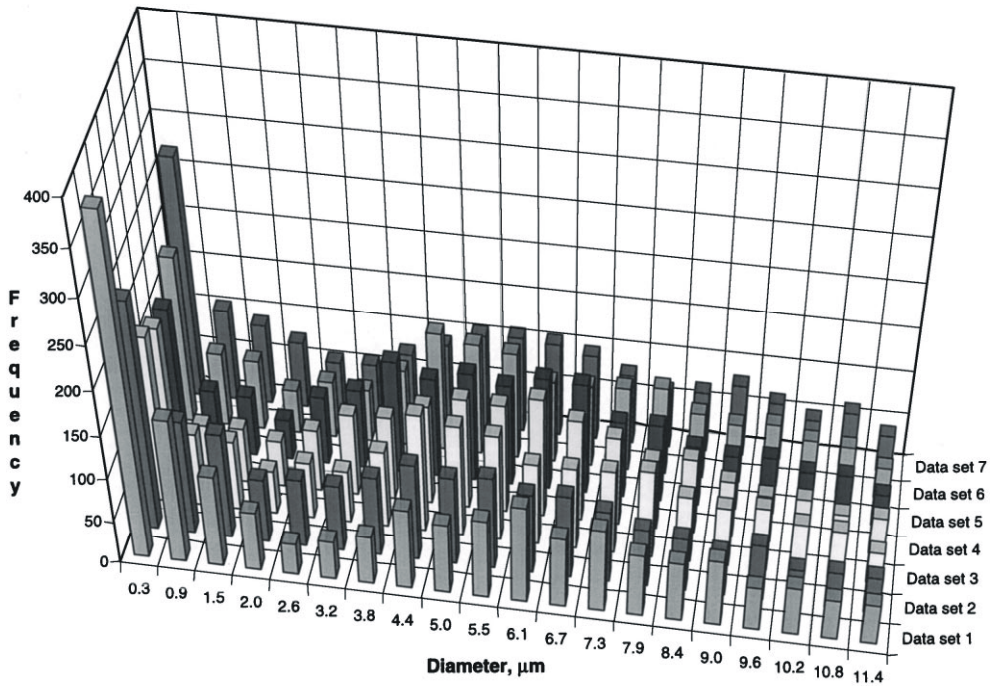

FIG. 4**.** *Size distributions of seven data for a sample, measured at different areas and times.*

What is the minimum number of particles to be analyzed to guarantee 5% reproducibility in CC EPXMA measurements?

The data measured for the sample prepared from bottle 8 were investigated to determine how many particles need to be analyzed to ensure the reproducibility at a certain level, for example, 5% RSD. Since particles in this sample are homogeneous in their sizes down to 15 ng (100 particles), each RSD for each chemical element was calculated for each data with a smaller number of particles down to 100, to apply the Ingamells sampling theory. If the morphology of the particles is homogeneous, which, for sure, is true for this sample down to 15 ng, the sample weight, w, in Eq. 2 can be converted into the number of particles analyzed. By fitting the data using Eq. 2 (an example of the results of fitting data is shown in Fig. 5.), the sampling constants, K_s for chemical elements are determined. Table VII shows those K_s values for the elements, and also the minimum number of particles needed to achieve 5% RSD in the measurements. The major elements in the sample, e.g. P, S, and K, show the smaller K_s values than those of the Mg, Ca, and Fe elements. For the minor chemical components, it is necessary to analyze more particles to get representative information on the sample. In Fig. 6, is shown the relationship between the number of particles needed to assure 5% RSD in the measurements and the average concentration of each element. Clearly, they are correlated; the less concentrated the element is, the more particles need to be analyzed for the analysis of the element. The number of particles required to be analyzed increases exponentially as the concentration of elements decreases.

TABLE V. RESULTS OF THE SMIRNOV TEST FOR SIZE DISTRIBUTIONS OF DATA FROM THE BOTTLE 8 TO THE FIRST DATA (SIGNIFICANT LEVEL P = 0.90 AND CRITICAL VALUE T= 0.24). THE NUMBER OF PARTICLES IN EACH DATA IS VARIED DOWN TO 50. DIFFERENT DISTRIBUTIONS FROM THE TARGET DISTRIBUTION OF THE FIRST DATA ARE SET IN BOLDS.

No. of particles	Data 2	Data 3	Data 4	Data 5	Data 6	Data 7
	Maximum difference between distributions					
2,000	0.08	0.16	0.19	0.19	0.16	0.09
1,800	0.08	0.16	0.19	0.20	0.09	0.09
1,500	0.07	0.15	0.17	0.17	0.09	0.08
1,200	0.09	0.16	0.19	0.19	0.11	0.11
900	0.08	0.17	0.20	0.20	0.10	0.11
600	0.15	0.18	0.23	0.21	0.12	0.12
300	0.08	0.22	0.19	0.20	0.10	0.14
100	0.13	0.14	0.13	0.14	0.16	0.10
50	0.14	**0.28**	0.18	**0.24**	0.12	0.12

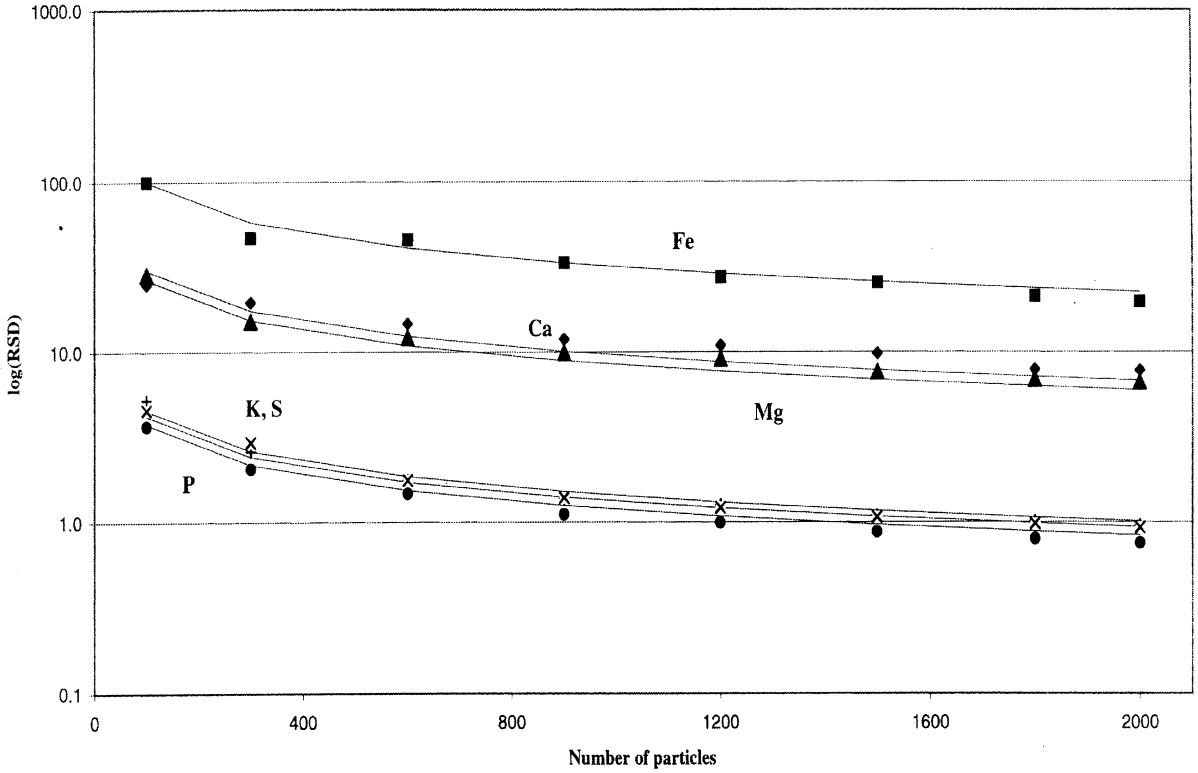

FIG. 5. *RSD vs. number of particles for each data. Ingamells sampling constant for each element is obtained by fitting data using Eq. 2. Resultant fitting curves are shown in solid lines. For the clearer display, RSD axis is in logarithmic scale.*

TABLE VI. RESULTS OF THE SMIRNOV TEST FOR COMPOSITIONAL DISTRIBUTIONS OF DATA FROM THE BOTTLE 8 TO THE FIRST DATA (SIGNIFICANT LEVEL P = 0.90 AND CRITICAL VALUE T = 0.24). THE NUMBERS OF PARTICLES IN EACH DATA ARE 100 (ABOUT 5 ng) AND 50 (ABOUT 8 ng). DIFFERENT DISTRIBUTIONS FROM THE TARGET DISTRIBUTION ARE SET IN BOLDS

Variable	Data 2	Data 3	Data 4	Data 5	Data 6	Data 7
	Maximum difference between distributions					
No of particles = 100						
Mg	0.07	0.03	0.05	0.05	0.04	0.02
P	0.07	0.07	0.18	0.09	0.11	0.07
S	0.09	0.10	0.15	0.12	0.07	0.10
K	0.15	**0.27**	**0.25**	0.21	0.19	0.15
Ca	0.03	0.08	0.06	0.04	0.02	0.04
Fe	0.05	0.05	0.03	0.05	0.03	0.01
No of particles = 50						
Mg	0.04	0.04	0.16	0.04	0.06	0.08
P	0.12	0.12	0.14	0.20	0.14	0.14
S	0.10	0.18	**0.30**	**0.22**	0.10	0.16
K	**0.40**	**0.40**	**0.44**	**0.34**	0.16	0.12
Ca	0.10	0.02	0.10	0.06	0.08	0.08
Fe	0.06	0.04	0.02	0.04	0.04	0.04

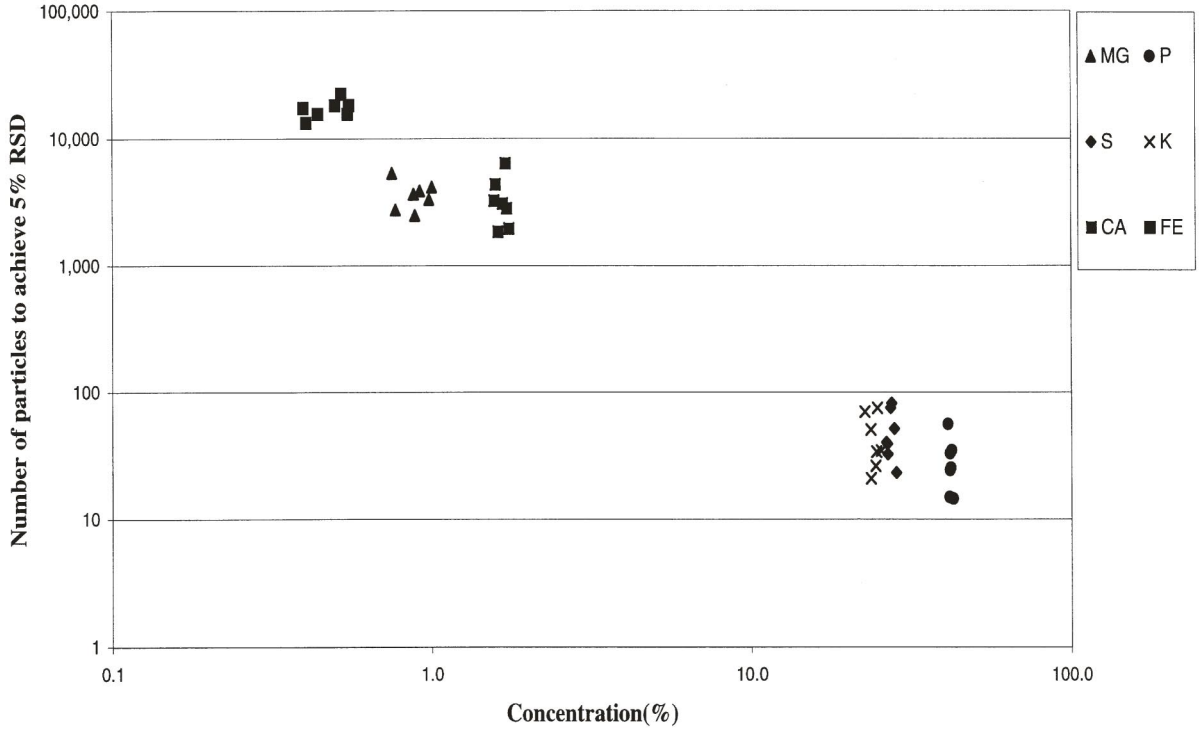

FIG. 6. *The relationship between the number of particles needed to assure 5% RSD and the average concentration of each element. The seven data for the same sample are used for the plot.*

TABLE VII. AVERAGE INGAMELLS SAMPLING CONSTANTS FOR ELEMENTS OBTAINED FROM ALL THE DATA AND AVERAGE NUMBER OF PARTICLES NEEDS TO BE ANALYZED TO ACHIEVE 5% RSD.

	Mg	P	S	K	Ca	Fe
Ks	101,949	3,409	3,308	3,954	76,839	392,073
5% RSD	4,078	136	132	158	3,074	15,683

Conclusions

At the level of ca. 300 ng, particle size distribution and distribution of concentrations of Mg, P, S, K, Ca, and Fe are the same for the samples from the different bottles of a IAEA candidate RM, except for bottle 40. One out of the remaining five samples, which are same at the 300 ng level, becomes different from the other four samples, when the sample mass is as much small as 8 ng.

The seven data measured at different areas and times for the same sample, are the same at the level of 300 ng, both in their sizes and compositions. At the level of 8 ng, two among seven data are different from the others, in terms of their size distributions. For the concentration distributions of the elements, four among the seven data are different at the level of 8 ng.

Even though the number of particles analyzed using CC EPXMA is very small compared to that collected, the major elements for this candidate RM need to be analyzed just for less than 200 particles, to assure 5% RSD in CC EPXMA measurements. For the minor elements, the required number of particles to be analyzed, for assuring 5% RSD, ranges from several thousands to tens of

thousands. The less concentrated an element is, the more particles are required to be analyzed for the element, to achieve meaningful reproducibility. The number of particles required to be analyzed, to insure a certain level of reproducibility, increases exponentially as the concentration of elements decreases.

ACKNOWLEDGEMENTS

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Role of NAA in determination and characterisation of sampling behaviours of multiple elements in CRMs

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Abtract. Taking the advantage of high precision and accuracy of neutron activation analysis (NAA), sampling constants have been determined for multielements in several international and Chinese reference materials. The suggested technique may be used for finding elements in existing CRMs qualified for quality control (QC) of small size samples (several mg or less), and characterizing sampling behaviors of multielements in new CRMs specifically made for QC of microanalysis.

INTRODUCTION

Minimum sample size (MSS), usually 150 mg, is the only parameter given in certificates of all existing biological and environmental (and most other) CRMs to describe sampling behaviors. Since the sampling behavior is very element-dependent, the general number, MSS, does not give information on sampling behaviors of individual elements.

CRMs certified at smaller sample size are required by QC of some modern analytical techniques (e.g. PIXE, micro-PIXE, solid sampling AAS, LAMMA, etc.) and some types of samples, the amounts of which available are very small (e.g. aerosols, monomineral grains, cosmic dust, some archaeological and forensic samples, etc.). They are also applicable and even preferable for many other modern analytical techniques (NAA, AAS, ICP-AES, ICP-MS, etc.).

Taking the advantage of high precision and accuracy of NAA in general, and that of hybrid K_0-relative NAA developed in our laboratory [1] in particular, Ingamells' sampling constants K_s [2] have been determined for multielements in IAEA RMs 396A/S and 396A/M, Air Particulate; Chinese Mn-Nodules RMs GSPN-2 and GSPN-3; and IAEA RM SD-M-2/TM Marine Sediment, and most recently IAEA-388 Lichen and IAEA-413 Algae, in an effort to cope with the above mentioned situation. According to Ingamells, a sampling constant K_s for a well-mixed material is defined as the minimum subsample needed to limit the relative sampling uncertainty to 1% at 68% level of confidence in a single determination, that can be expressed by the following equation:

$$K_s=R^2w \qquad (1)$$

where, R^2 is relative sampling variance (68% confidence level) in decimal function, determined from the analysis of a set of subsamples with the weight of w each.

Visman's double sampling constants, A and B, [3] have also been used for elements with large K_s values in IAEA RM SD-M-2/TM for segregation evaluation. A and B are expressed by the following equation:

$$S^2=A/nw + B/n \qquad (2)$$

where, S^2 is relative sampling variance for the average of n subsamples with each weight of w; A and B are homogeneity constant and segregation constant, respectively.

Multielements have also been determined in IAEA-388 and IAEA-413 on 150 to 200 mg sample size by hybrid k_0-relative NAA.

Multielements have also been determined in IAEA-388 and IAEA-413 on 150 mg sample size by hybrid k_0-relative NAA.

EXPERIMENTAL

Preparation of Samples and Standards

Two to three mg each of 12 to 15 subsamples for each of the five RMs studied (IAEA 396A/S, 396A/M; Chinese GSPN-2, GSPN-3; IAEA SD-M-2/TM; IAEA-388 and IAEA-413) were weighed and wrapped in PE bags (for short irradiation) or Al foil (for long irradiation). Chemical standards of elements to be determined were prepared. Twenty five μm thick Zr foil was used as neutron flux ratio monitor, and weighed high purity Fe wire as comparator for K_0-NAA. NBS SRMs 1632a and 1633a were used as QC materials.

For IAEA SD-M-2/TM, a set of large size samples, about 150 mg each, was prepared for the determinations of Visman's double sampling constants for selected elements.

Regular procedures were used for determinations of multielements in IAEA-388 and IAEA-413.

Irradiation and Counting

All irradiations were conducted at the heavy water research reactor (HWRR) of our Institute. Thermal neutron fluxes are 3×10^{13} n/cm^{2}s for long irradiation and 1×10^{13} n/cm^{2}s for short irradiation, respectively. After irradiation, the samples and standards were transferred into PE counting vials. All countings were carried out with a HPGe gamma ray spectrometer (Canberra, 26%, 2.0 keV). Neutron flux variations over each irradiation package were previously checked to be within 1%.

Data Reduction

In NAA, the hybrid K_0-relative NAA software, ADVNAA [1], was used for calculations of elemental concentrations.

In calculations of Ingamells sampling constants, the following equations were used:

$$K_s=R^2w$$
$$R^2=S_o^2-S_a^2 \quad (3)$$
$$S_a^2=S_c^2+S_w^2+S_g^2+S_f^2 \quad (4)$$

where, S_o -- observed relative standard deviation over a set of sub-samples;
S_a -- relative analytical uncertainty;
S_c -- relative counting statistics;
S_w -- relative uncertainty in weighing;
S_g -- relative uncertainty in counting geometry;
S_f -- relative uncertainty in neutron flux.

In calculations of Visman's double sampling constants A and B, the following equations were used:

$$S_{sm}^2 = A/nw_{sm}+ B/n \quad (5)$$
$$S_{lg}^2 = A/nw_{lg}+ B/n \quad (6)$$

By solving the above simultaneous equations, we have

$$A=w_{sm}w_{lg}(S_{sm}^2-S_{lg}^2)/(w_{lg}-w_{sm}) \quad (7)$$
$$B=S_{sm}^2-A/w_{sm} \quad (8)$$

where, subscripts sm and lg stand for small size of sub-samples and large size of sub-samples, respectively.

RESULTS

K_s and A,B values for IAEA RM SD-M-2/TM are listed in Table 1. K_s values for Chinese RMs GSPN-2,3, IAEA RMs 396A/S, 396A/M and IAEA-388 and 413 are given in Tables 2, 3 and 4, respectively. Analytical results (on 150 mg sample size) for 36 elements in IAEA-388 and 26 elements in IAEA-413 are listed in Table 5.

DISCUSSION

i) Parametric normalization for different counting positions using the EID principle, developed in our laboratory [5], has made it possible to use conventional size of existing CRM samples (150 to 200 mg, specified in certificates) for QC in analysis of micro-samples (<5 mg).

ii) The hybrid extended K_0-relative NAA method maximizes the number of the determinable elements (all elements detected can be determined), and provides an internal validation tool for detecting and reducing the systematic errors relative to calibration.

iii) Sampling behaviors are very element-dependent. The minimum sample size given in existing CRMs, usually 150 mg, is too conservative for many elements (K_s<150 mg) in the five RMs studied, and not large enough for some other elements, such as Tb, Yb, Lu in IAEA SD-M-2/TM; Au, Hf, Lu in IAEA 396A/S and Au in IAEA 396A/M.

iv) Five to ten elements are homogeneous (relative sampling uncertainty less than 1%) at 5 mg sample size in the five RMs studied.

v) The relation between particle size and sampling behavior can be clearly observed from K_s values for IAEA 396A/S and 396A/M, which are actually the same material with different particle sizes (maximum particle sizes are 40 μm and 10 μm for IAEA 396A/S and 396A/M, respectively). The most distinguishable differences in sampling behaviors occur in Au, Cr, Hf and Th with Ks values of 12000, 100, 750 and 100 mg for 396A/S and 170, <2, 50 and 4 mg for 396A/M for those four elements, respectively.

vi) The fact that the elements with K_s<5 mg are often scattered in atomic number in the CRMs studied makes it possible for those CRMs to be used for QC in thick target PIXE, which has effective sample size of 2 to 7 mg (for Na to Th) in silicate matrices with target diameter of 6 mm. For example, in IAEA 396A/M eleven elements (Ba, Br, Co, Cr, Fe, La, Na, Sb, Sc, Th, Zn), with rather evenly distributed atomic numbers from 11 (Na) to 90 (Th), have K_s values of 2 to 6 mg, well qualified as a first ever CRM of its kind for thick target PIXE.

vii) Segregations were found for some elements in IAEA SD-M-2/TM by using Visman's double sampling constants (B>0).

viii) The suggested technique may be used for finding elements in existing CRMs qualified for QC of small size samples, and characterizing sampling behaviors of multielements in new CRMs specifically made for QC of microanalysis.

TABLE I. K_S AND A,B VALUES FOR IAEA SD-M-2/TM[4]

Element	K_s, mg	A, mg	B	Element	K_s, mg	A, mg	B
Br	12			Lu	390		
Ce	70			Mn	12		
Co	<1			Na	<1		
Cr	27	24	1.9	Sc	<1		
Cs	<1			Sm	120	115	1.0
Eu	90	86	0.5	Tb	1500		
Fe	<1			Th	65	60	1.9
La	65	50	5.1	Yb	800		

TABLE II. K_S VALUES FOR CHINESE RMS GSPN-2,3, MG

Element	GSPN-2	GSPN-3	Element	GSPN-2	GSPN-3
As	80	110	Na	5	<2
Ba	58	46	Sb	8	<2
Ce	36	<2	Sm	5	3
Co	3	<2	Sc	<2	3
Eu	15	16	Th	42	3
Fe	<2	5	Yb	30	9
La	<2	<2	Zn	106	93
Lu	110	52	Mn	<2	9

TABLE III. K_S VALUES FOR IAEA RMS 396A/S, 396A/M, MG

Element	396A/S	396A/M	Element	396A/S	396A/M
As	40	30	La	120	6
Au	12000	170	Lu	280	80
Ba	20	5	Mn	110	50
Br	<6	6	Na	6	2
Ca	60	20	Rb	30	13
Ce	100	<25	Sb	2.5	<1
Co	11	6	Sc	5	4
Cr	100	<2	Sm	100	20
Cs	20	20	Ta	30	30
Eu	120	60	Th	100	4
Fe	<1	<1	Zn	6	3
Hf	750	50			

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Analysis of micro matter reference materials of lichen and algae by SRXRF and PIXE

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Abstract. In the present work two nuclear micro analysis techniques, synchrotron radiation (SRXRF) and macro proton induced X ray emission (macro PIXE), were used in the homogeneity test of Algae IAEA-413 and Lichen IAEA-338, and the certification of their elemental contents too. Finally, the Ingamell's sampling constant Ks and the relative homogeneity factor H_E of some elements in these two RMs were estimated on the base of our macro PIXE results.

1. Introduction

Analytical quality assurance (QA) is essential for a reliable chemical analysis. Many Certified Reference Materials (CRMs) from various producers are commonly used in trace elemental analysis for methodological development and quality control purposes. Normally most of existing standard reference materials (SRMs) were certified with the minimum sample mass larger than 100 mg. Some modern analytical techniques are quite sensitive and only very small amount of sample is requested in the measurements, especially in some nuclear analytical techniques in which the analyzed mass of sample could be as low as mg, μg or even down to ng level**.** Due to heterogeneous distribution of trace components in many reference materials (RMs) and other present constituents which interfere the results, it is often very difficult to directly use these RMs in the micro analytical techniques. The analysts, who are working in micro analytical laboratories, are interested in having some micro matter CRMs for better quality control and accuracy improvement. The homogeneity and accurate certified values of various chemical compositions of SRMs are expected for the application of micro nuclear analytical techniques. The efforts to produce new SRMs in excellent homogeneity and to determine the certified values with improved techniques have been making in some laboratories. On the base of past experiences the International Atomic Energy Agency (IAEA) has initially produce reference materials for micro analytical nuclear techniques. Algae material IAEA-413 and Lichen material IAEA-338 are two kinds of these new RMs.

2. Experimental

2.1 Sample preparation

According to the IAEA guide procedures, 0.3-0.4 gram of the two RMs in each bottle (No. 006, 014, 022, 030, 038, 046 of Algae IAEA-413 and No. 13, 17, 46, 86, 126, 166, 196 of Lichen IAEA-338,) was weighed and the bottle was immediately closed after sampling, and then the same operation were done every time of weighing. The samples were dried in a oven for 6 h at 80°C. The piled sample thickness in the container for drying was about 3 mm. The dried samples were immediately placed in a desiccator with fresh silica gel. The samples were weighed after the following 8 h in order to establish temperature equilibrium.

The dried sample powders were pressed into pellets in diameter 13 mm with a 5 ton press. All the pellets were reserved in a desiccator with fresh silica gel.

2.2 SRXRF analysis for homogeneity test

The homogeneity of all the sample pellets were tested by the SRXRF probe. It was performed on the Beijing Synchrotron Radiation Facility (BSRF) together with the Beijing Electron Positron Collider (BEPC) built in our Institute of High Energy Physics, the Chinese Academy of Sciences. The energy of BEPC was 2.204 GeV and its beam intensity and luminosity was 95.4 mA and $10^{30}/cm^2 \cdot sec$, respectively. Our measurement was carried out on 3W1A, one of beam lines in BSRF. The energy of synchrotron radiation was in the range of 3.5-22 KeV (after a beryllium window). The maximum acceptance angle was 1.0 mrad (horizontal) and 0.1 mrad (vertical). The beam spot sizes could be adjusted from 20 μm to 200 μm. Combining SRXRF with a high precision specimen's scanning stage, which is home made and can be operated with three dimension displacement and one rotation, it can be used for micro beam computerized topography and scanning X-fluorescence analysis. At the present work the size of micro-probe was confined to 20 μm×20 μm, the displacement precision was 1 μm and the rotation angle was 1' per step.The X ray spectra were detected by a Si(Li) detector mounted at 90° to the beam direction. The area of the detector is 30 mm^2 and its resolution is 134 eV for 5.95 KeV. In order to evaluate the homogeneity level in micro area, 9 spots were scanned along the diameter (13 mm) of a target pellet, each in size of 20 μm×20 μm, 500 μm apart from each other. All the spectra of SRXRF were analyzed with AXIL computer code [1].

2.3 Macro PIXE analysis of elemental contents of RMs

There is a Van de Graaff accelerator with 2.5 MV in our laboratory. We were not satisfied with its lower yields of heavy elements in PIXE measurements. For improvement we made macro PIXE measurement with a Pelletron tandem accelerator, model 5SDH-2 (2 × 1.7 MV) in the Department of Technical Physics, Peking University.

The macro PIXE measurements were performed under vacuum condition in a PIXE target chamber using 3.0 MeV proton beam of 3 mm diameter. The PIXE spectrum was measured by a Si(Li) detector covered with a 12.5 μm beryllium window and its resolution is 175 eV for 5.95 KeV. In order to improve the minimum detection limitation (MDL) of the trace elements in target sample, the target chamber was well designed and the Si(Li) detector was mounted in 135° to the proton beam direction and 22.5° to the normal direction of the target. The beam intensity irradiated on the target was adjusted to about 1 nA. The count rate was around 300 CPS and the preset charge was about 1 μC. A funny Mylar absorber of 450 μm thick with a 0.7% hole fraction of the Si(Li) detector area was placed in front of the Si(Li) detector to improve the detection limits of both light and heavy elements in the target samples.

The macro PIXE spectra were analyzed with GUPIX computer software package[2-3], which is specifically suitable for analying PIXE spectra of thick specimens. The software provides nonlinear least-squares fitting of the spectrum, together with subsequent conversion of the fitted X ray peak intensities to elemental concentrations via defined standardization technique involving fundamental parameters and user-determined instrumental constant.

In order to get the best accuracy of the quantitative PIXE analysis, samples have to be homogenous with a flat surface at the beam spot region. The knowledge of matrix composition is necessary for thick target analysis. As for the major chemical composition of the analyzed sample, the data was kindly offered by Dr. M. Jaksic in Croatia in private communication. He mentioned: " Since the analyzed thickness by PIXE depends on the X ray energy of the element analyzed, we did calculate this on the basis of GUYLS program (in GUPIX package) in the approximation of the sample thickness (in mg/cm^2) for which 90% of the yield comes. For the approximate matrix component of Algae and Lichens which are very similar we obtained by RBS approximation: 55% C, 37% O, Na 1%, P 2%, K 1%, Ca 2%, S 2%. This approximation is very good and does not influence the results very much. "

In our case two SRMs (Milk Powder IAEA-A11 and Whey IAEA-155), which are very fine powders and suitable for use with ion beam analysis (IBA) because of their nature, were used as standards and analyzed by macro PIXE to test the fundamental parameter approach by GUPIX in our laboratory. Basing on the comparison between the measured contents and the certified values for SRMs, IAEA-A11 and IAEA-155, it is evident that the fundamental parameter approach by GUPIX for macro PIXE analysis in our laboratory is acceptable.

3. Results and discussion

3.1 Homgeneity of the RMs

The peak areas of the X rays for measured elements in each irradiated spot region were divided by the peak area of argon in air to normalize the SR irradiated on a spot of target pellet. For homogeneity test the normalized peak areas of the feasible elements in 9 spots of each target pellet were determined for Lichen IAEA-338 and Algae IAEA-413. As shown in the tables the relative standard deviations (RSD=S.D./ mean value) of elements K, Ca, Mn, Fe, Cu, Zn, Br, Rb and Pb for Lichen IAEA-338, and K, Ca, Cr, Fe, Ni, As, Hg and Pb for Algae IAEA-413, are less than 10%. However, for other elements their RSDs are worse. The tables show the average values of normalized elemental peak areas of the samples from each bottle, the total average value of each material, and their relative standard deviations for IAEA-338 and IAEA-413, respectively. The results show that these two RMs are promising ones with quite good homogeneity.

3.2 Determinated values of elemental contents of the RMs

All the target pellets sampled from the 7 bottles of Lichen IAEA-338 and the 6 bottles of Algae IAEA-413 were measured by macro PIXE. Two of the PIXE spectra of the lichen and algae samples are shown in Fig. 4 and 5, respectively. Table 6 and 7 list the elemental contents of the samples from each bottle of Lichen IAEA-338 and Algae IAEA-413, which were analyzed using the fundamental parameter approach. It is shown that many elements such as Ca, Mn, Fe, Cu, Zn, Br and Pb for Lichen IAEA-338, and Ca, Cr, Mn, Fe, Ni, Cu, Zn, As, Br and Pb for Algae IAEA-413 have good RSDs, which are not bigger than 10%.

3.3 Sampling constants and homogeneity factor

According to Ingamells [4], a sampling constant can be given as

$K_s = R^2 * m$

where K_s = Sampling constant, R = relative standard deviation, m = mean sample mass (mg).

Stoeppler et al. [5] took the square root of this factor to calculate a relative homogeneity factor H_E:

$H_E = S_{HOM} * \sqrt{m}$

where H_E = relative homogeneity factor, S_{HOM} = relative standard deviation, m = mean sample mass (in mg).

According to Dr. M. Jaksic group's results, the analyzed thickness by PIXE depends on the X ray energy of the element analyzed, they calculated this on the basis of GUYLS program in the approximation of the sample thickness for which 90% of the yield comes. Using their results of areal density (in mg/cm^2) depent on the atomic number Z for 90% yield, We can calculate the mass m (in mg) for our beam spot size of 0.0707 cm^2.

The Ingamell's sampling constant Ks and the relative homogeneity Kurfurst factor H_E can be calculated from our macro PIXE analysis. It is shown in the tables that K_s and H_E are in the range of 10^1 and 10^0 order level for about 7 (Ca, Ti, Mn, Fe, Cu, Zn, Pb) and 8 (K, Ca, Cr, Mn, Fe, Ni, Cu, Zn) feasible elements of IAEA-413 and IAEA-338 samples, respectively. As Kurfurst pointed out in his original papers [5-7], a $H_E < 10$ points to very good homogeneity. Far less than 10 mg sampling mass and the homogeneity level shown in the tables indicates that these two RMs are promising SRMs for qualitative and quantitative analysis of micro amount samples.

Although it is difficult to accurately calculate Ks and H_E of IAEA-338 and IAEA-413 with SRXRF micro probe analysis without the knowledge of relationship of the areal density and sample mass for 90% yield. Still they could be estimated basing on " the analyzed thickness depends on the X ray energy of the element analyzed ". Since the irradiated spot size in SRXRF measurement was 20 μm × 20 μm = 4×10^{-6} cm^2, which is about 5×10^{-5} times in PIXE measurement, the ratio of RSD in SRXRF to that in PIXE is less than 4 times in our case, H_E for many elements in the two RMs using SRXRF should be one third of those using macro PIXE, i.e. in the order of 10^0 or even down to 10^{-1} level. These two materials have H_E values in this range and can be considered as SRMs for micro-analytical techniques with a sampling mass between 10 mg and 0.1 mg order level for analysis. As can be seen from above results, both SRXRF and macro PIXE are suitable nuclear techniques for relative homogeneity test and the elemental contents certification. SRXRF has better minimum detection limit (MDL) for middle and heavy elements, whereas PIXE has better MDL for middle and light elements. It is better for scientists, who are interested in biological and environmental studies, to analyze and assess toxic elements in specimens using SRXRF.

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Studies of some IAEA candidate reference materials for microanalytical techniques

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Abstract. In order to develop new reference materials for microanalytical nuclear techniques, the Scanning Proton Microprobe (SPM) technique was used to determine homogeneity level within 100 × 200 μm^2 micro-area on the small pieces of IAEA Urban Dust reference materials. In part 1 of this paper, the experimental methods are described in detail. The results show that IAEA-396A/M Vienna Urban Dust is homogeneous enough for small sample analysis. As a task we prepared the IAEA-386 bovine liver as a new candidate reference material to meet this purpose. In part 2, the preparation process including material collection, dried, pulverize, sieve, homogenization and preliminary test is described in detail. The more effective grinding methods were established to achieve the median particle size of 22 μm. Also in part.3 we performed the qualitative determinations of some candidate reference materials by NAA and AFS.

Introduction

Standard Reference Materials (SRMs) are an indispensable element of quality assurance. They play a key role in demonstration of accuracy of analytical work. Up to now, the reference materials are mostly satisfactory for various analytical techniques, but there is an increasing demand for micro quantitative information of nuclear analysis techniques. Most RMs are certified for minimum sample sizes larger than 100 mg by producers. A minimum sample size, which is compatible with the respective analytical technique, however, is one of the most important requirements for a suitable RM. Therefore RMs with such large sample sizes are useless for methods such as XRF, NAA, PIXE and other accelerator-based methods, which commonly use and analyze samples in the mg mass range or even smaller samples. The CRP organized by IAEA specifically addresses the question of quality control materials for micro-analytical nuclear techniques.

Part 1

Scanning proton microprobe microanalysis for the assessment of homogeneity of IAEA urban dust reference materials

Experimental Method

In order to assess distribution of trace elements and micro-homogeneity to smaller samples, some Urban Dust powder reference materials 396A/M and 396AiS were put into clean cups and dried at 85^0C for twenty-four hours. Then 160 mg samples of both powder reference materials were weighed and Ø 13 mm diameter small pieces were prepared by pressing these samples in a 10 ton press. Measurement is performed utilizing the Scanning Proton Microprobe experimental set-up in our institute as shown in fig. 1.

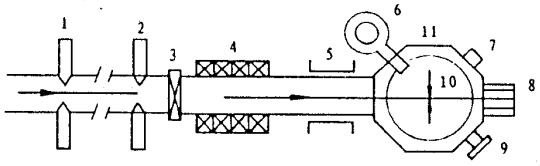

FIG. 1: *A diagram of the scanning proton microprobe 1: object; 2: aperture; 3: vacuum valve; 4: quatrupole lenses; 5: deflect. Coils; 6: Si(Li) detector; 7: window; 8: faraday cups; 9: vacuum pump; 10: sample target; 11: target chamber.*

It employs an NEC 4MV pelletron accelerator as an ion beam injector. The proton microprobe is a Russian quadruplet constructed with four magnetic quadruples. The focal length of the microprobe line is greater than 40 cm and the overall length of the microprobe line is about 9 m, in order to achieve a demagnification of 20 times. The beam size is around 2 μm and the current on the sample is about 10 PA. The vacuum target chamber is an octagonal construction. A retractable 28 mm^2 Ortec Si(Li) detector covered with a thin beryllium window is mounted at 135^0 to the beam direction. The scan size in this experiment is 100 × 200 μm^2 as shown in fig. 2. A multiparameter multichannel analyzer ND-76 is used for event by event data collection and a Micro-VAX computer system is employed data treatment.

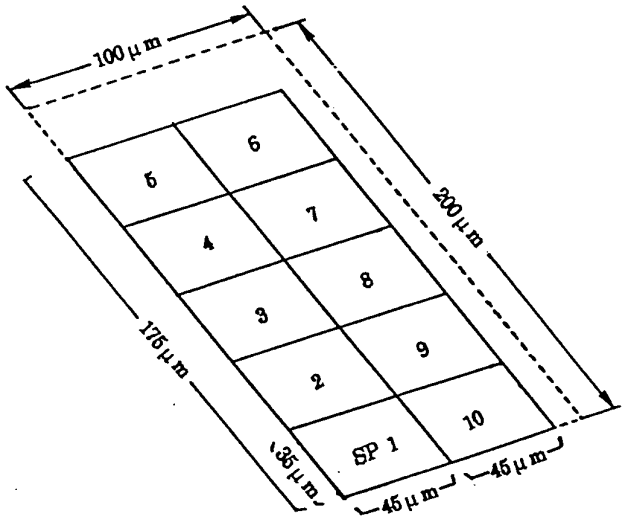

FIG. 2: *A scanning region of micro- PIXE.*

Results and Discussion

Fig.3 is a typical point PIXE spectrum of Vienna Urban Dust IAEA-396A/M. In order to evaluate the homogeneous level quantitatively in more micro area, we device the scanning region 100 × 200 μm^2 into ten micro areas, each 35 × 45 μm^2, as shown in Fig.2. The average results of ten micro PIXE intensity maps for each element are shown in table 1. Also the same results are more directly displayed in two pictures of fig. 4. The elements of As and Kr are under detective limit, so their data do not be included in table 1. Among the two fractions of the Vienna Urban dust IAEA-396 SRM, final fraction sample shows higher degree of homogeneity. From the X ray intensity maps, very weak inhomogeneity is visible only for Cr and Ti. Coarse fraction has more signification inhomogeneity, visible for Al, Si, S, Cl, Ti and Cr. In the concentration data for the fine fraction sample just elements Ti and Cr have results scattered more than *25%* and these are probably caused by insufficient statistics.

A sophisticated graphic program is built in to display elemental maps in the form of three-dimensional isometric and two or three-dimensional contour maps. It is interesting to see three-dimensional distributions of elements in two kinds of Vienna Urban Dust in order to compare the particle size homogeneity. In fig 5 is shown three-dimensional distributions of representative three elements within 100 × 200 μm^2 pressing pieces of 396A/M and 396A/S respectively. The contour maps are more favorable than other maps because from them one can get both qualitative information on distribution profile and quantitative information on elemental intensity and localization. From the pictures, it can be clearly seen that the homogeneous level in 396A/M is better than that in 396A/S. These results are also in accordance with fig.4.

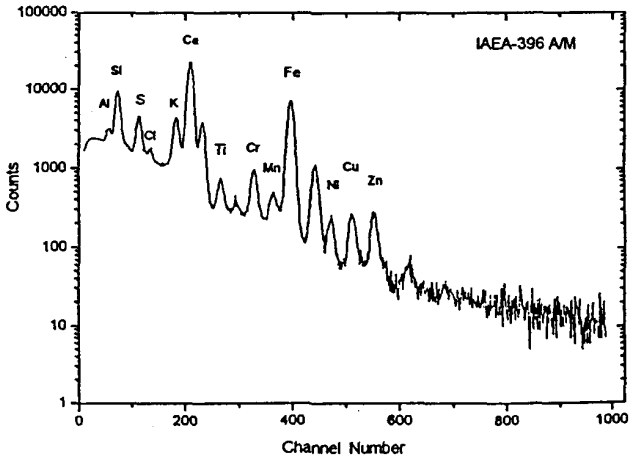

FIG. 3: *A typical point PIXE spectrum of Urban Dust IAEA 396/A/M.*

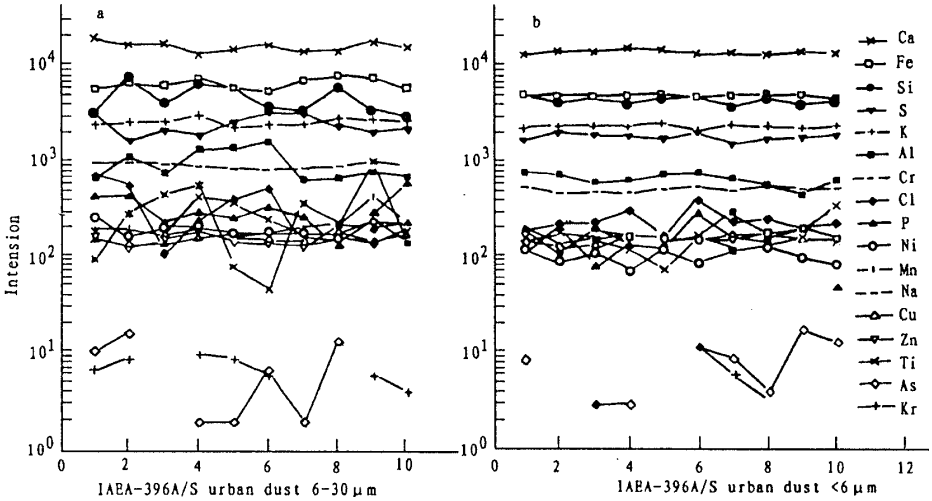

FIG. 4: *Results in ten spectra of IAEA urban dust.*

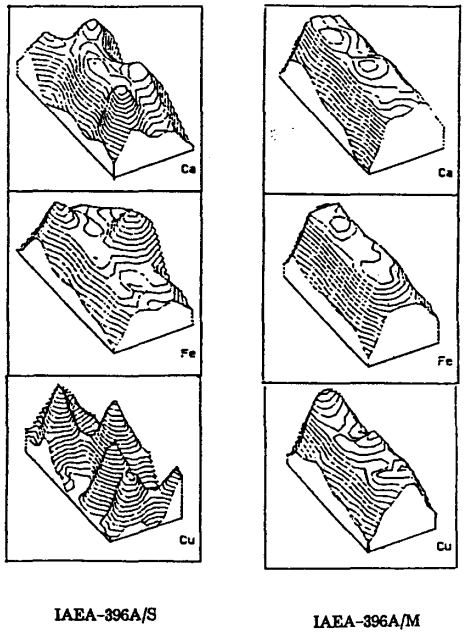

FIG. 5: *Distribution maps of elements in the scanning region 100 X200 mm2 of Vienna urban dust IAEA-396A/M and IAEA-396A/S.*

Based on the total mass and the area of a Ø 13mm diameter small piece we can roughly estimate the mass of scanning region 35 45 μm^2 as 2μg. It is far less than analysis samples in the mg mass range goal. We can see that IAEA-396A/M has very good homogeneity level at such low scale and is suitable for possible certification at the 1 mg level. To sum up, by the development of focused proton or X ray beams, the quantitative analysis of elemental distribution in the micro masses (go down to μg or even ng level) can be obtained. Also the advantage of the SPM PIXE technique is possibility of monitoring homogeneity of all elements in the sample by means of the X ray intensity maps across the scanned region.

Table I. Micro-PIXE Results of IAEA-396A Urban Dust (net area)

Element	IAEA-396A/M Average X	Std.Dev. σ	Rel.Dev. σ/X	IAEA-396A/S Average X	Stad.Dev. σ	Rel.Dev. σ/X
Al	591	88	15	860	297	35
Si	4392	443	10	4687	1523	32
S	1822	187	10	2461	664	27
Cl	251	52	25	314	185	59
K	2314	116	5	2586	256	10
Ca	13636	763	5	15882	1851	12
Ti	194	71	36	319	307	96
Cr	463	177	38	769	198	26
Fe	5083	183	3	6738	809	12
Ni	107	18	17	207	30	15
Cu	158	12	7	160	18	12
Zn	164	17	10	160	29	18

Part 2

Preparation of Bovine Liver Candidate Reference Material

Experimental Method

Material collection

Fresh bovine livers were collected from normal male calves (just born a week) in Second Animal Farm, Shanghai suburb, in 1996. The average wet weight of each fresh bovine liver is about 800 gram. Approximately 83 Kg of sample including in total 104 bovine liver individuals was kept in clean polythene bags and stored in low temperature refrigerator (bellow -40^0C) until preparation.

To keep mineral contamination and the loss of mineral elements from the samples to a minimum, instruments used for the collection of specimens and their handing were acid cleaned and washed with high purity water. All operations were performed in a special cleaning-room to avoid any contamination of the material with metals.

Cleaning and drying

First we take out the sample from refrigerator to thaw them at normal temperature (10–20 ^{0}C) for 24 hours. After removal of storage bags and visible contaminants, all liver samples were rinsed by high purity water to remove blood or surface fluid drainage to obtain the wet weight. Then we cut the liver tissues into small pieces with titanium knife and removed the blood tube at the same time. Later we beat these liver tissues using food pulverizer with titanium spinning knife. Approximately 63 Kg homogeneous mixture of liver tissue was obtained and placed in plastic drum for keeping.

The drying of samples was performed using freeze-drying machine in Shanghai Biological Production Institute. After thawing, the liver mixture was placed on aluminum trays, which were put into the machine and dried at -40^0C for 35 hours, Finally the liver mixture was reached constant weight. The ratio R=0.23 of dry to wet weight was determined, and about 16 kg of dried liver was obtained.

Pulverize and sieve

The grinding of the dried liver tissue into powder was accomplished by using a agate ball mill pulverizer (QM-1 SP. produced by Instrument Factory of Nanjing University, China). This mill machine is designed by planet principle. Forty agate balls of 20 mm and 10 mm diameters were put inside of each agate pot. Four agate pots in all were mounted on the machine plate and revolved round the main axis on their own axis in the opposite direction. The rotational speed is adjustable from 50-300/mm.

The bovine liver powder was sieved through 80 μm plastic nylon sieve. The fraction retained on the sieve was returned to agate pots for further grinding again. The ground and sieved material about 12Kg was collected in a plastic drum for further treatment.

Homogeneity and particle size determination

The homogeneity, which is one of the basic requirements for a candidate reference material can be achieved by a thorough mixing of the powdered material. The bovine liver powder was blended in two ways to achieve the fine powdered material. First, the fractions were put into a polyethylene rotation drum, which was placed in a specially constructed homogenizer. It is able to rotate in two directions thus assuring good mixing of the material. Then the mixing material was transferred to a Y type homogenizer with Teflon lined (produced by Japan), and blended automatically for more than 3 hours to achieve a high degree of homogeneity.

The determination of particle size distribution in powder liver using Mastersizer X (Malvern Instruments Ltd.) laser light scattering instrument in connection with a dry powder sampling unit. The mass of the sample analyzed was approximately 40 mg in each case. Four samples in all were performed particle size distribution measurements and results were shown in a cumulative graph as Fig 6. We can see that an average of the median particle's diameter for four samples is about 22 μm and the size corresponding to the largest peak in the distribution is about 35 μm.

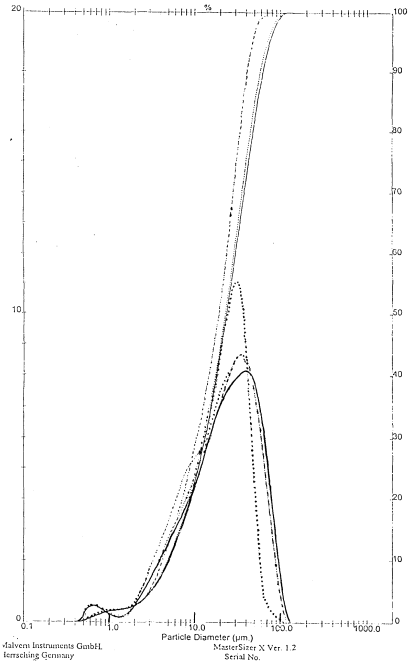

FIG. 6: *Particle size distribution in sample of bovine liver.*

Conclusion

Bovine liver powder has been prepared as a part of CRP reference materials for microanalytical nuclear techniques. The results show that its particle size distribution was improved significantly than these of other RMs. For reasons of quality control and better assessment of resulting data, its certification campaigns on world-wide will be organized by IAEA.

Acknowledgments

The authors wish to acknowledge Dr. R. Zeisler for his helpful suggestions and thank Dr. A. Fajgelj for allowing us to use his measurement report of bovine liver particle size.

Part 3

Determinations of some IAEA candidate reference materials by NAA and AFS

Experimental method

The qualitative analysis of some micro amount samples was performed in my institute. 10mg samples of powder IAEA-338 lichen, IAEA-413 algae and IAEA-386 bovine liver were weighed and put into a small clean polyethylene envelope for NAA analysis. Slowpoke reactor and NAA experimental set up were built in Shanghai Analysis Survey Center. Three groups of each ten samples were successively placed in a pneumatic transfer rabbit system and irradiated for 10 min at a thermal neutron flux of 8x 10^{11} n/cm^{2}sec in the reactor. All samples were analyzed under the same conditions (10 min irradiation, 3 min decay and 5 min count). The activities of some low Z elements like ^{27}Mg, ^{56}Mn, ^{24}Na, ^{52}V, ^{28}Al, ^{38}C1, ^{66}Cu, ^{49}Ca, etc were measured by using an Ortec Ge(Li) detector having a resolution of 2.1 Kev of ^{60}Co ray. The detector was coupled to a 8192 channel pulse height analyzer equipped with PC computer system. The net peak area and error were printed out automatically based on the NAA program.

The analysis of As, Se and Hg in algae and lichen was performed by method of Atomic Fluorescent Spectrometer (AFS).

Analysis results

Table II. NAA results of IAEA-413 Algae (μg/g)

No	Al	Ca	Cl	Mg	Mn	Na	V
A1	84.4	2674	601.4	3974	163.1	363.6	1.33
A2	92.8	3029	673.8	3894	156.7	344.5	1.46
A3	88.8	2687	670	3730	162.4	346.2	1.49
A4	93.9	2512	682.6	3938	165.3	364.9	1.31
A5	84.5	2594	661.8	3852	162.4	351.9	1.27
A6	89.1	3069	642.9	3789	168.4	350	1.45
A7	84.9	2849	701.6	4031	168.5	362	1.47
A8	85.6	2915	701	3689	154.2	354.6	1.48
A9	99.5	2910	664.5	4040	158.4	360.7	1.41
A10	85.9	3047	652.5	3696	160.8	365.7	1.38
average	88.9	2828.6	665.2	3863.3	162.0	356.4	1.41
stdev	5.0	199.8	29.3	133.3	4.7	8.0	0.08
RSD	5.7	7.1	4.4	3.5	2.9	2.2	5.60

Table III. NAA results of IAEA-338 (μg/g)

No	Al	Br	Cl	I	Mg	Mn	Na	V	Ca
L1	481.9	17.1	2362	2.99	394.4	54.5	121.7	3.88	3216
L2	491.7	19.5	2061	3.63	624.6	47.4	131.1	3.61	3240
L3	517.3	17.2	2045	3.18	612.3	45.9	133.6	3.78	3304
L4	474.8	18.7	2038	3.44	442.8	45.5	124.3	3.5	3081
L5	471.8	18.1	2063	2.73	638.2	50.4	130.2	3.29	3361
L6	488.7	18.7	2065	2.88	666.2	48.3	129.8	3.19	3339
L7	474.2	17.9	2060	3.54	464.9	45.1	135.2	3.26	2898
L8	475.4	19.6	1980	3.74	436.2	47	126.7	3.77	2970
L9	479.2	18.9	2050	3.4	477.8	47.5	130.4	3.1	3236
L10	493.7	20	1989	3.39	618.8	44.9	138.6	3.54	3180
average	484.9	18.6	2071.3	3.29	537.6	47.7	130.2	3.49	3182.5
stdev	13.8	1.0	106.6	0.33	102.8	2.9	5.0	0.27	154.5
RSD	2.8	5.3	5.1	10.17	19.1	6.1	3.9	7.79	4.9

Table IV. NAA results of IAEA-386 Bovine Liver (μg/g)

No	Br	Cl	Cu	Mg	Mn	Na
B1	12.7	3967	456.4	758.8	7.06	3252
B2	8.95	4019	479.5	756.6	7.28	3371
B3	10.8	3841	467.1	801.3	7.24	3356
B4	12.6	4034	456.1	802	6.91	3283
B5	17.7	3987	462.8	685.3	7.72	3387
B6	8.13	3889	476.6	774.1	7.09	3297
B7	12.8	4004	419.9	771.2	7.25	3292
B8	11.9	3899	414.8	736.7	7.25	3407
B9	14.5	4044	418.2	680.1	6.68	3392
B10	12.5	3863	462.8	764	6.96	3280
average	12.3	3954.7	451.4	753.0	7.1	3331.7
stdev	2.7	75.1	24.5	42.0	0.3	56.4
RSD	22.0	1.9	5.4	5.6	3.9	1.7

Table V. AFS results of IAEA-338 and IAEA-413 (μg/g)

Sample No.	IAEA-338			IAEA-413	
	As	Se	Hg	As	Hg
1	0.84	0.19	0.28	155	46.6
2	0.74	0.3	0.39	139	48.4
3	0.72	0.19	0.21	144	48.2
4	0.59	0.2	0.33	134	47
5	0.76	0.24	0.23	157	47.3
6	0.58	0.19	0.23	144	47.8
7	0.63	0.19	0.33	152	47.3
8	0.63	0.29	0.25	146	47.3
9	0.78		0.32	143	47.7
10	0.67		0.33	157	48.6
average	0.69	0.22	0.29	147	47.6
stdev	0.09	0.05	0.06	7.87	0.64
RSD	12.6	21.1	20.2	5.3	1.3

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Characterisation of candidate reference materials by PIXE analysis and nuclear microprobe PIXE imaging

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Abstract. In order to test weather some candidate reference materials show homogeneity that can satisfy quality control of the PIXE technique, six bottles of each of the two Candidate RM's — Lichen (IAEA 338) and Algae (IAEA 413) were tested. Four different tests were performed. First, two pellets from each bottle were prepared and analysed using broad beam (ϕ=5 mm) PIXE. Second and third was analysis of homogeneity using scanning focussed beam at the nuclear microprobe. Scans of 50x50 μm^2 and 240x260 μm^2 were performed. Finally, individual grains with composition differing from the rest of the sample, were analysed using PIXE and RBS.

1. Introduction

Similarly to XRF (X ray Fluorescence) spectrometry, broad beam PIXE (particle induced X ray emission) analysis has been for decades successfully used as an analytical technique for determination of minor and trace element concentrations in various sample matrices. Sample mass portions typically analysed by all X ray emission spectrometry techniques (XRF, PIXE, EPXMA, etc.) are in the range between ng (microprobe) and mg (broad beam) level. Although not certified for such small sample sizes, numerous powder reference materials (mainly produced by IAEA) were successfully used world-wide in many XRF and PIXE laboratories. Development of focussed beams (electrons, protons or photons) that interact with much smaller sample masses (pg, ng), increase the need for reference materials with sufficient homogeneity on much lower mass scale than certified in past (grams). IAEA has recentely produced several materials homogeneous on much lower mass scale. Vienna urban dust (fine fraction), Algae and Lichen are some of these materials.

2. Experimental set-up

The most important technical consideration in the measurements of inhomogeneities by PIXE or micro-PIXE is the problem of spectra normalisation. Namely, errors in beam charge measurement (secondary electrons, statistical fluctuations, ground loops, etc.), dead time correction imperfections, or geometrical changes (sample to detector distance, target inclinations), increase the final systematic error in data. As a result, concentrations of all elements in particular sample are over or under estimated. In such conditions, standard deviation of the average concentration values for a large series of similar samples is dominated by large systematic errors.

In order to improve this aspect of PIXE analysis, the IRB PIXE chamber has been upgraded with additional direct and indirect charge measurement systems (see Figure 1). In addition to this, simultaneous RBS analysis was used for a further correction of total collected charge.

Summary of all measurements performed to find the best reproducibility for our PIXE set-up is given in Table 1. It is seen that for samples having different matrix (various biological CRMs), secondary electron suppression improves the results. However, for the series of samples with equal matrix (algae 292 and 293), standard deviation does not change. Further measurements on Algae 413 and Lichen 338 (which are presented here) were unfortunately dominated by erratic changes in detector dead time. In order to be able to use collected spectra, renormalisation of all results onto the fixed value for potassium was therefore applied. Potassium was used due to the good homogeneity (as observed by microPIXE) and high counting statistics in the X ray peak

Much simpler way of achieving equal conditions for PIXE analysis could be used in nuclear microbeam PIXE analysis. A single large scanning area was divided in a definite number of equal areas. Instability in charge collection, geometrical imperfections and dead time correction is in this case equal for all selected regions and therefore more reliable comparison can be achieved. Schematic presentation of IRB nuclear microprobe set-up is given in Figure 2.

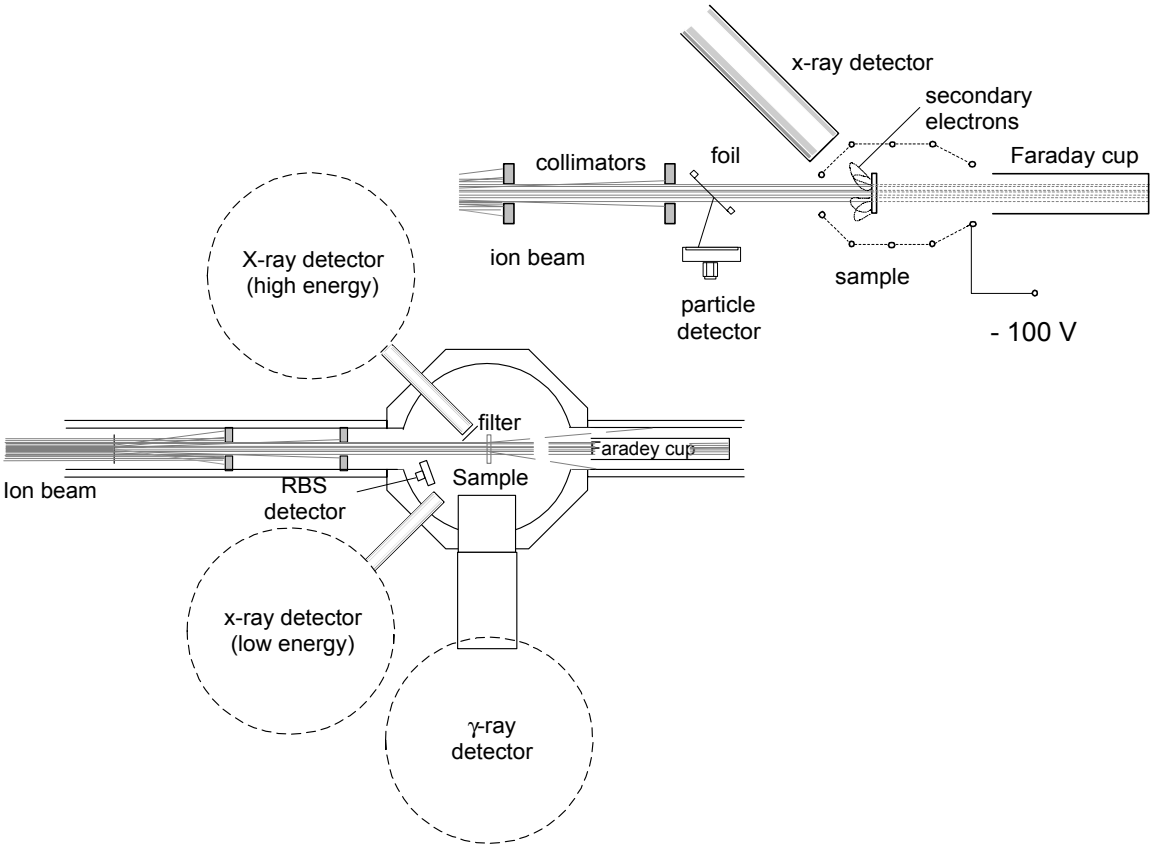

FIG 1. *Schematic presentation of the IRB PIXE set-up (lower left) that comprises of two Si(Li) X ray detectors (low energy — small solid angle without filter, high energy — large solid angle and filter), intrinsic Ge γ ray detector and PIPS particle detector. Alternative solutions used for the current measurement are shown in the upper right corner. Indirect charge measurement is made using the backscattering from the thin metal foil, while the direct charge measurement is done by a charge digitiser connected to the sample frame and Faraday cup which are surrounded by a secondary electron emission suppression system.*

TABLE I. VALUES FOR STANDARD DEVIATION (σ_R(%)) OF THE SERIES OF ANALYSES (N IS NUMBER OF INDEPENDENT MEASUREMENTS), FOR DIFFERENT SAMPLES AND EXPERIMENTAL CONDITIONS.

	supress	N	H-value	K	Ca	Cr	Mn	Fe
biological RMs	-	12	13.1					
biological RMs	+	6	6.9					
Algae 292	-	11		5.1	4.4		7.7	5.4
Algae 293	-	12		2.5	3.6	3.9	8.7	6.6
Algae 293	+	10		5.8	4.7	7.1	10.7	6.2
Algae 293 (K normalized)	+	10		0	3.4	3.0	6.4	4.8

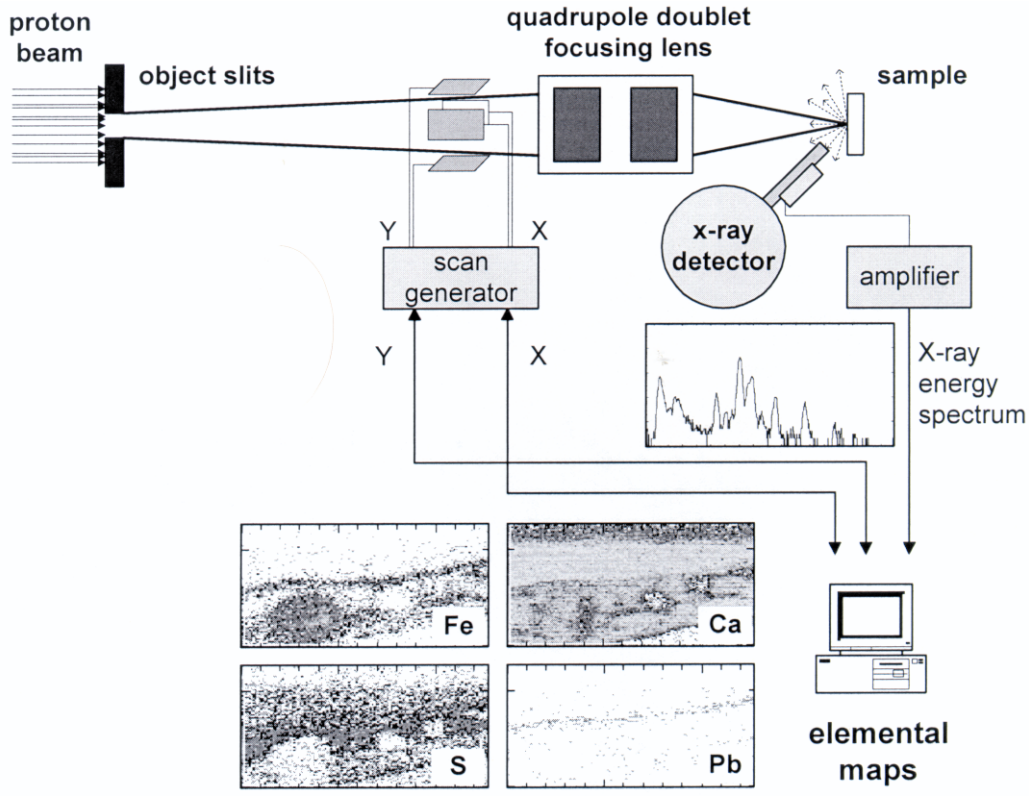

FIG 2. *Schematic presentation of the IRB nuclear microprobe PIXE set-up.*

3. Homogeneity of Algae 413 and Lichen 338 Candidate RMs

Six bottles of the two candidate reference materials (Algae 413 and Lichen 338) were used in the investigations. Two pellets from each bottle were prepared for broad beam PIXE analysis, while only two pellets of each Candidate RM were analysed by microPIXE.

Since the highest contribution to the X ray intensity comes from the surface sample layers, their here is no straightforward relationship for the analysed sample mass. Therefore we calculated sample thickness that contribute to the 90% of X ray yield (Figure 3). The sample area that was exposed to the beam was then multiplied by this thickness to obtain the "sample mass".

All results are given as the relative standard deviations σ_R (contribution from the inhomogeneity) that is derived from the measured total standard deviations and its analytical contribution according to:

$$\sigma_T^2 = \sigma_R^2 + \sigma_A^2$$

3.1. PIXE broad beam analysis (CRMs homogeneity at the mg level)

For the broad beam PIXE analysis a 3 MeV proton beam of currents around 1 nA was used. Total accumulated charge for each sample was 1μC. A high solid angle, 80 mm^2 Si(Li) detector was used for the detection of X rays without any filtering. Such conditions were favourable for the low energy X rays. Since sufficient statistics could be obtained only for these X rays, results were given only for elements between Si and Fe.

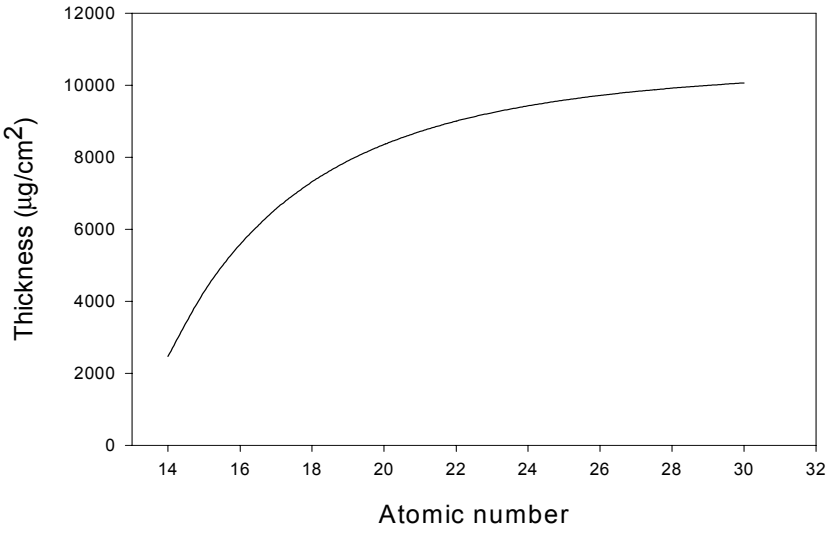

FIG. 3. *Thickness of the biological matrix sample that corresponds to the thickness of the sample slab that contribute to the 90% of the K X ray yield for the elements of atomic number 14>Z>30 excited by 3 MeV protons.*

As it was indicated previously, spectra were normalised on the same potassium concentration. Standard deviation was obtained after subtraction of counting statistics and spectrum fitting errors. In Table 2 and Table 3 results for both materials are given. In the case of Algae 413 (Table 2) it is seen that standard deviation for all presented elements is bellow 3%. Counting statistics error for chromium was larger then the experimental one.

For the second Candidate reference material Lichen 338, the only element with standard deviation above 5% is silicon. All other elements show very good homogeneity. As it is shown in the tables, sample mass for the studied elements ranged between 1 and 2 mg.

TABLE II. RESULTS OF ANALYSIS (CONCENTRATIONS ARE GIVEN IN PPM) FOR THE ALGAE 413 SAMPLE. SAMPLE NUMBERS INDICATE THE BOTTLE NUMBER.

	S	**K**	**Ca**	**Cr**	**Fe**
047B	3281	3465	939	139	513
047A	3211	3465	950	128	517
007B	3177	3465	879	143	546
007A	3174	3465	903	138	551
031A	3196	3465	909	135	520
039B	3180	3465	930	127	551
$C_{av.}$ (ppm)	3203	3465	918	135	533
σ_I(%)	1.27	0	2.85	4.71	3.40
mass (μg)	1096	1550	1640	1850	1907
σ_R(%)	0.98		2.36	-	2.67

TABLE III. RESULTS OF ANALYSIS (CONCENTRATIONS ARE GIVEN IN PPM) FOR THE LICHEN 338 SAMPLE. SAMPLE NUMBERS INDICATE THE BOTTLE NUMBER.

	Si	**S**	**Cl**	**K**	**Ca**	**Fe**
192B	3450	2224	3598	5039	6576	1745
192A	3747	2336	3688	5039	6528	1675
013B	4096	2373	3782	5039	6648	1639
013A	4103	2405	3766	5039	6665	1664
081B	3891	2405	3705	5039	6679	1629
082A	4049	2339	3745	5039	6494	1610
122B	4284	2492	3838	5039	6544	1652
122A	3929	2277	3602	5039	6427	1675
162A	4525	2557	3813	5039	6404	1652
$C_{av.}$ (ppm)	4008	2379	3726	5039	6552	1660
σ_T(%)	7.70	4.30	2.30	0	1.53	2.31
mass (μg)	484	1096	1289	1550	1640	1907
σ_R(%)	**7.55**	4.07	2.12	-	1.44	0.69

3.2. Nuclear microprobe PIXE imaging (CRMs homogeneity at the μg level)

Development of microprobe techniques in X ray analysis (nuclear, X ray and electron microprobes), increased the need for reference materials homogeneous at μg level sample sizes. Due to its scanning capability, nuclear microprobes can on-line image sample inhomogeneity. As it will be showed here, microPIXE images can be used to image very small inhomogeneities. In order to perform this, candidate RMs was scanned in two different scan areas. In addition to images of elemental distribution that immediately showed homogeneity or inhomogeneity, each X ray intensity map was divided in twelve regions of the same size for the further reproducibility evaluation.

Standard deviation for each element was calculated by subtracting the contribution of the counting statistics. For samples scanned over 1200 × 600 μm^2 area, division to 12 regions of 200 × 300 μm^2 area was performed. Sample mass for these measurements was in the range between 2 and 6 μg. In order to investigate homogeneity on even smaller sample masses, further reduction of scan size to 200 × 300 μm^2 was performed. By dividing this area to 50 × 50 μm^2 regions, sample masses were reduced to 60–250 ng.

Results of the Candidate RM Algae 413 are presented in Table 4 and 5. It is seen that for all elements apart iron and manganese, standard deviation is bellow 2%, which indicate very good homogeneity. Even in the case of iron, standard deviation is better than 10%.

In Figure 4 elemental distributions for potassium and iron in the 1200 × 600 μm^2 area of the pellet sample of the Algae 413 Candidate RMs are presented. Corresponding statistical analysis is given in Table 4. Inhomogeneity, or contamination it this sample is clearly seen. In this and similar cases it was clear that microPIXE imaging can provide much faster and more reliable evidence about the inhomogeneity.

TABLE IV. RESULTS OF THE NUCLEAR MICROPROBE PIXE SCAN OVER THE PELLET OF THE ALGAE 413 SAMPLE. THE TOTAL SCAN WAS DIVIDED TO 12 REGIONS OF 200 × 300 μM^2. X RAY INTENSITIES FOR ELEMENTS WITH SIGNIFICANT STATISTICS ARE GIVEN IN COUNTS FOR THE EACH REGION.

sample	P	S	K	Ca	Cr	Mn	Fe
1	54106	40448	46321	16729	1258	563	2151
2	52961	40808	45943	16400	1270	617	2140
3	53752	41312	46088	16806	1329	603	2230
4	52814	40374	45751	16258	1246	595	2070
5	54603	41762	46388	16754	1243	615	2005
6	54400	42146	47093	16717	1292	650	2193
7	53350	40632	45766	16750	1283	621	2228
8	53165	40891	45618	16526	1258	572	2128
9	53870	40860	45861	16508	1284	628	2281
10	52932	40520	45068	16656	1249	714	2351
11	54180	41194	46346	16958	1284	671	2866
12	53542	41225	45853	17298	1280	606	2367
$I_{av.}$ (counts)	53639	41014	46008	16696	1273	621	2250
σ_T(%)	1.13	1.32	1.09	1.61	1.91	6.70	9.82
mass (μg)	2.56	3.35	4.74	5.01	5.66	5.75	5.82
σ_R(%)	1.04	1.22	0.98	1.41	-	**5.37**	**9.59**

TABLE V. RESULTS OF THE NUCLEAR MICROPROBE PIXE SCAN OVER THE PELLET OF THE ALGAE 413 SAMPLE. THE TOTAL SCAN WAS DIVIDED TO 12 REGIONS OF 50 × 50 μM^2. X RAY INTENSITIES FOR ELEMENTS WITH SIGNIFICANT STATISTICS ARE GIVEN IN COUNTS FOR THE EACH REGION.

sample	P	S	K	Ca	Cr	Mn	Fe
1	29707	22145	24512	8614	755	302	1151
2	29669	21876	24329	8554	724	269	980
3	30474	21833	24338	8491	759	293	856
4	30052	21908	24522	8649	708	302	948
5	30427	22544	24736	8575	773	271	934
6	30345	21937	24399	8436	737	286	905
7	30332	22182	24102	8561	713	273	903
8	29865	21959	24683	8380	725	281	840
9	29922	22629	25220	8779	774	251	894
10	30869	22533	24854	8540	691	280	911
11	30262	22204	24517	8456	749	265	885
12	29941	22159	24186	8358	750	286	961
$I_{av.}$ (counts)	30155	22159	24533	8532	738	280	931
σ_T(%)	1.18	1.25	1.25	1.39	3.56	5.41	8.64
mass (μg)	0.11	0.14	0.19	0.21	0.23	0.24	0.24
σ_R(%)	1.03	1.06	1.08	0.87	-	-	**8.00**

TABLE VI. RESULTS OF THE NUCLEAR MICROPROBE PIXE SCAN OVER THE PELLET OF THE LICHEN 338 SAMPLE. THE TOTAL SCAN WAS DIVIDED TO 12 REGIONS OF 200 × 300 μM^2. X RAY INTENSITIES FOR ELEMENTS WITH SIGNIFICANT STATISTICS ARE GIVEN IN COUNTS FOR THE EACH REGION.

sample	**Si**	**Cl**	**K**	**Ca**	**Fe**
1	7194	7989	9619	12119	756
2	8026	7874	9780	11345	855
3	7972	7756	9534	11296	814
4	8162	7731	9166	10904	956
5	7463	7837	9302	11462	891
6	8216	7885	9560	11537	856
7	7390	7930	9487	11671	859
8	7696	8165	9793	11861	781
9	7714	8363	10053	11948	845
10	8296	8476	9724	11544	795
11	8455	8080	9790	12047	880
12	9126	8273	9801	12233	795
$I_{av.}$ (counts)	7975.8	8029.9	9634.1	11663.9	840.2
σ_I(%)	6.67	3.02	2.52	3.36	6.60
mass (μg)	0.062	0.164	0.197	0.209	0.243
σ_R(%)	**6.57**	2.81	2.30	3.22	**5.62**

TABLE VII. RESULTS OF THE NUCLEAR MICROPROBE PIXE SCAN OVER THE PELLET OF THE LICHEN 338 SAMPLE. THE TOTAL SCAN WAS DIVIDED TO 12 REGIONS OF 50 × 50 μM^2. X RAY INTENSITIES FOR ELEMENTS WITH SIGNIFICANT STATISTICS ARE GIVEN IN COUNTS FOR THE EACH REGION.

sample	**Si**	**Cl**	**K**	**Ca**	**Fe**
1	14733	13511	15701	18417	1498
2	15178	13339	15603	17786	1407
3	15017	13442	15486	17639	1427
4	15468	13262	15667	18008	1432
5	15344	13315	16946	18297	1453
6	17141	13631	17982	18527	1522
7	14276	12943	15324	17503	1386
8	14405	13138	15273	17272	1262
9	14177	13366	15730	18168	1412
10	15110	13493	16090	18267	1409
11	14618	13735	15935	18113	1451
12	15106	13691	16103	18182	1449
$I_{av.}$ (counts)	15047	13405	15986	18014	1425
σ_I(%)	5.20	1.71	4.82	2.14	4.52
mass (μg)	1.48	3.94	4.74	5.01	5.83
σ_R(%)	**5.13**	1.48	4.75	2.01	3.66

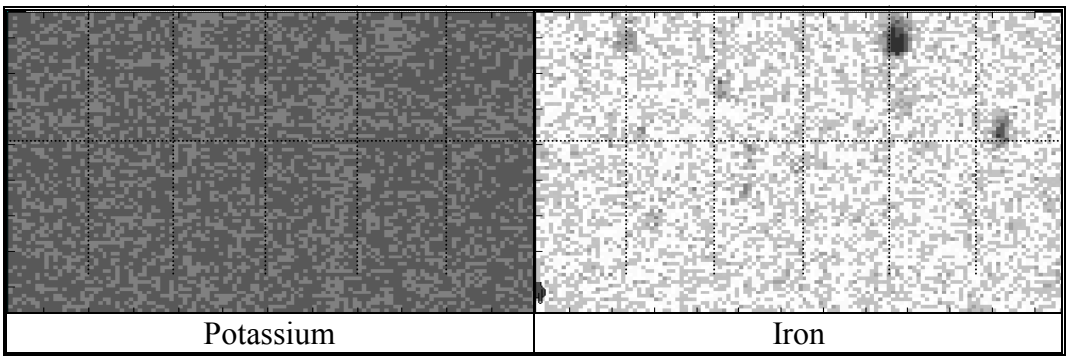

FIG 4. *MicroPIXE image of the Algae 413 sample for which the quantitative results are given in Table 4.*

3.3. Characterisation of contaminants

As it was shown previously, microPIXE imaging can provide information about particular inhomogeneities. If the microbeam is positioned at the point or scanned only over particular region of interest, PIXE spectrum of this particular point or region can be measured. In Figure 6 a and b, we are presenting analysis of iron rich contaminants in algae sample. As it is seen from the spectra, in those two cases high iron concentrations is correlated by high manganese or titanium contents.

In the case of more severe contamination cases, RBS analysis can be used for the characterisation of matrix composition of particular contaminants. It is seen from figure 5 that contaminants in algae are having the same matrix composition as the sample bulk.

4. Discussion

In order to be able to clearly state the level of particular element homogeneity, sufficient counting statistics in PIXE measurements have to be obtained. Therefore, we establish criterion that the relative analytical standard deviation σ_A has to be below 5%. In such conditions (visible from Tables), contribution of the sampling standard deviation σ_R to the experimental (total) standard deviation σ_T is dominating one. In Figures 7 and 8, results of the dependence of σ_R on the estimated sample mass are shown. Expected behaviour that homogeneity is increasing with sample mass can be seen for chlorine, calcium and iron in Lichen sample. The other results (perhaps due to errors in determination of σ_R) do not show such behaviour.

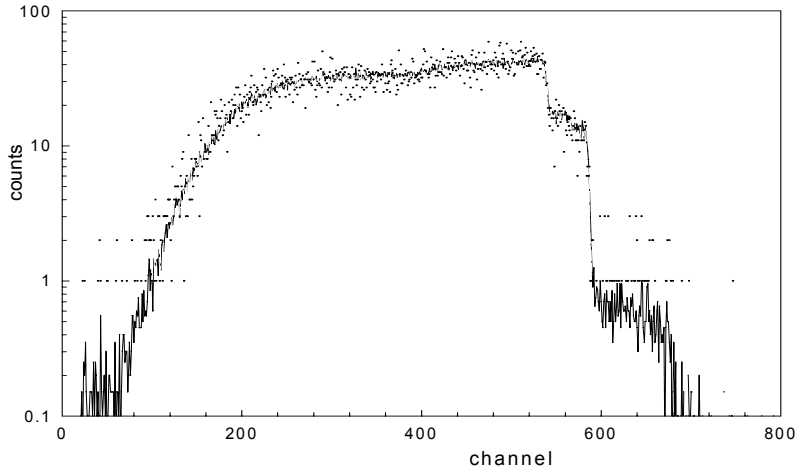

FIG 5. *RBS spectrum of the Algae sample (solid line) and contamination zone from the Figure 6b (dotted line). No significant differences in matrix composition are visible for this particular example.*

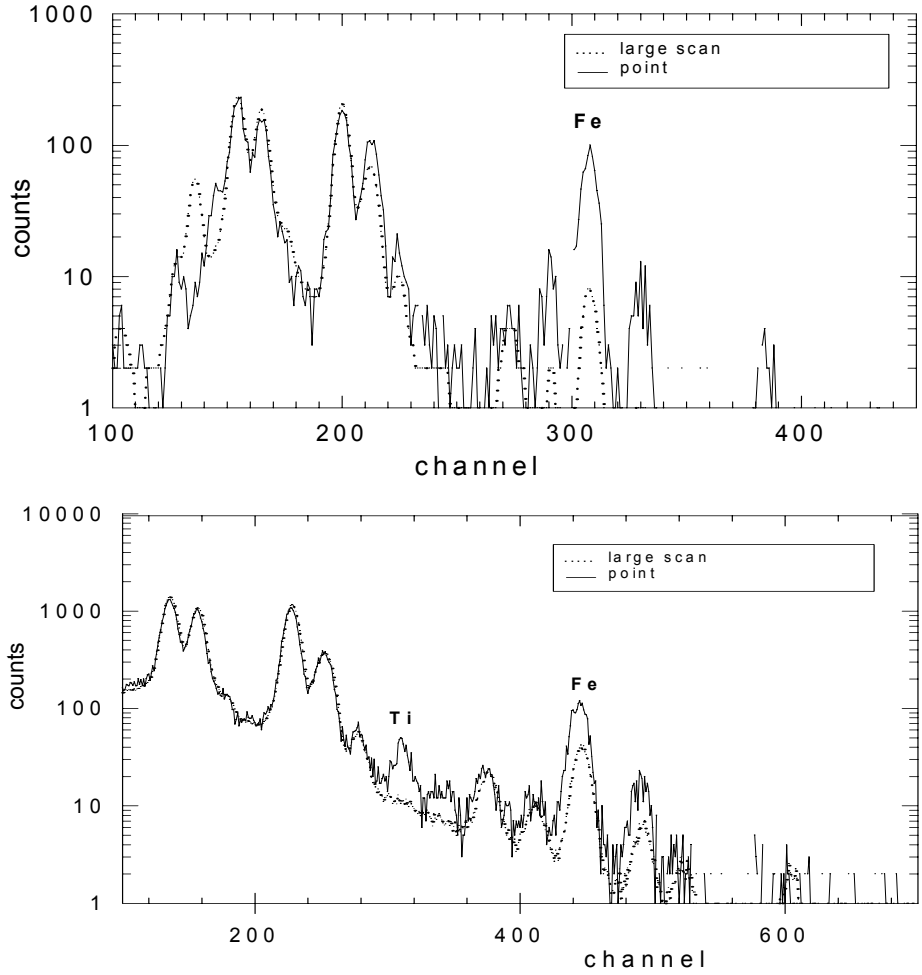

FIG 6a (UP) AND 6b (DOWN). PIXE spectrum of two observed inhomogeneities in the Algae sample.

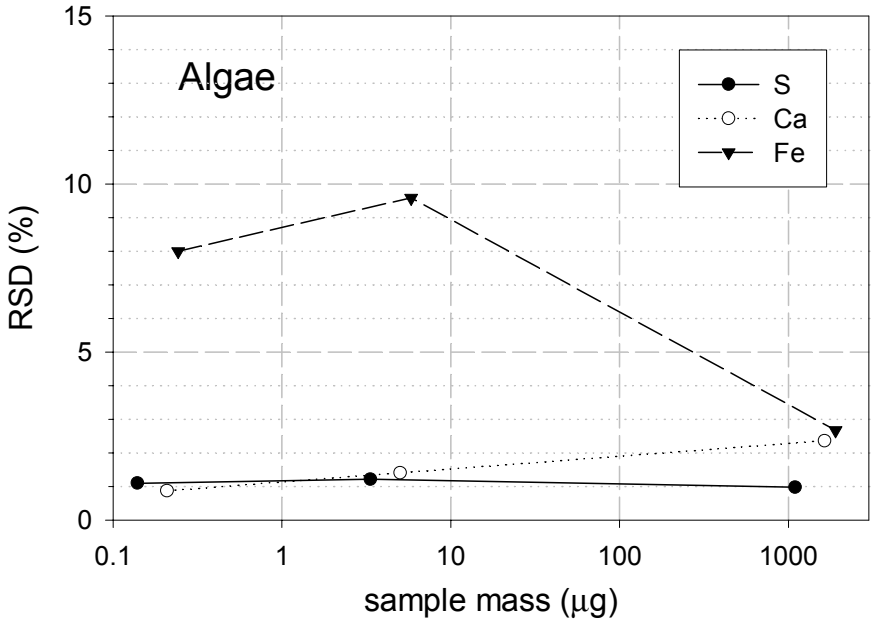

FIG 7. Relative sampling standard deviation for three elements in Algae 413 as a function of sampling mass.

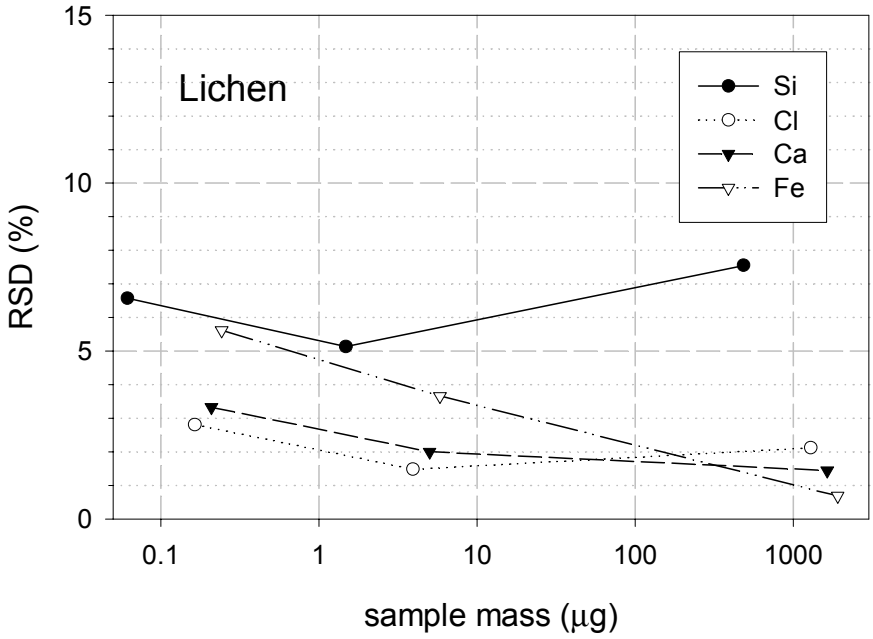

FIG 8. *Relative sampling standard deviation for three elements in Lichen 338 as a function of sampling mass.*

Testing the homogeneity of candidate reference materials by solid sampling — AAS and INAA

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Abstract. The necessity to quantify a natural material's homogeneity with respect to its elemental distribution prior to chemical analysis of a given aliquot is emphasised. Available instruments and methods to obtain the relevant information are described. Additionally the calculation of element specific, relative homogeneity factors, H_E, and of a minimum sample mass $M_{5\%}$ to achieve 5% precision on a 95% confidence level is given. Especially, in the production and certification of Certified Reference Materials (CRMs) this characteristic information should be determined in order to provide the user with additional inherent properties of the CRM to enable more economical use of the expensive material and to evaluate further systematic bias of the applied analytical technique.

Introduction:

Direct analytical techniques, such as Instrumental Neutron Activation Analysis (INAA) and Solid Sampling-Atomic Absorption Spectrometry (SS-AAS) face a rapid development towards specific applications in chemical analysis where other methods, depending on the dissolution of solid materials cannot easily compete. Due to their superior power of determination, absence of losses and contamination caused by chemical manipulation of the sample, and due to the small sample mass consumed for analysis these techniques offer particular opportunities to solve some analytical problems otherwise hard to attack. During the last three decades a large variety of applications of SS-AAS has been reported in the literature [1]. Newly emerging needs in various fields of analytical chemistry suggest that direct, fast and cost-effective solid sample analysis is a challenging alternative to the dissolution methods; particularly if a solid sampler is combined with a multi-element detector, such as an atomic emission- or a mass spectrometer (AES or MS).

A comprehensive overview on the history, the development and the particular advantages of direct and slurry sampling using GF-AAS and ETV-ICP techniques has been published recently [2]. A great number of references demonstrate advantageous applications of direct solid sampling analysis. To mention just a few the outstanding power of determination was emphasised by the analysis of impurities in high purity refractory materials such as ZrO_2, GaAs, high purity quartz, and high purity Ta, Ti, Al_2O_3 or graphite powder [3,4,5,6]. The speed to obtain analytical results is clearly shown by emergency actions in environmental accidents such as the Thallium analysis in the surrounding of a cement factory or the detection of mercury in sediment after an accidental spill of a chemical plant to the river Rhine. For analytical product control of industrial processes fast and reliable results are contributing to the product quality and considering legal limits, they may prevent financial losses [7,8,9,10]. In food industry, where a delay in the production due to slow analytical methods can influence the composition of ingredients, direct solid sampling analysis should be the method of choice [11,12].

The low sample mass consumed by SS-AAS (0.05 to 50 mg) is a prerequisite for the investigation of element distributions in biological tissues, toxicological and biomedical studies and in forensic analysis. The determination of Ni in single strands of human hair to assess the time of exposure [13] might not that easily being repeated by an analytical method based on the digestion of

samples. Trace element distribution in saliva [14] and small kidney stones as well as in biopsy samples is preferentially carried out on mg-samples using the direct analytical approach without any dilution of the original material. In forensic studies the direct analysis of sub-mg gun shot residues could help to clarify the assault in several murder cases [15,16].

Another important application of SS-AAS taking advantage of the small sample mass used for analysis is homogeneity testing and quality control in the production process of certified reference materials (CRMs) [17,18]. Heterogeneity of natural, solid materials with regard to their chemical composition of different degrees seems to be a natural phenomenon. Chemical analysis, however, using a small probe of the entire lot is depending on a certain degree of homogeneous composition in order to extrapolate from the analytical result of the small aliquot to the whole lot correctly. Therefore, before accurate analysis can be carried out, natural solid materials (e.g. environmental, biological, medical, geological samples etc.) need to be physically homogenised by grinding, sieving and mixing.

It is a challenging task to characterise and quantify the degree of homogeneity in a certain material. The larger the number of individual particles in a certain mass aliquot, the higher the probability is to determine equal concentrations of analytes in subsequent aliquots. A qualitative estimation of homogeneity, hence, can be carried out by the determination of the particle size distribution of the processed material.

A problem arises when materials of completely different composition are merged together, such as geological and biological (e.g. earthworms having soil ingested in the gut, biological tissues with calcified inclusions etc.). In these cases even careful grinding and sieving might not produce the degree of homogeneity needed for reliable and precise analysis of some elements. Very large aliquots need to be processed carefully to obtain accurate results.

The preferential way to determine the degree of homogeneity of an element in a material is by repetitive analysis of a large number of small solid aliquots. Direct solid sample analysis as carried out by INAA and SS-AAS was used because chemical dissolution of a larger aliquot of the solid material destroys the heterogeneity characteristics and may cause unexpected contamination or losses. Additionally, heterogeneity of well- mixed solid materials generally is detectable only on a small sample mass range (low mg). Digestions for dissolution analysis in most cases are, however, carried out on 100–500 mg aliquots. Here SS-AAS and INAA demonstrate some of their most powerful advantages: high sensitivity and stability, small enough sample mass, and speed of analysis (and for INAA multielement capability).

Instruments used for homogeneity determination:

For about 20 years the only commercially available direct solid sampling AAS instrument based on Zeeman effect background correction was provided by a small German company, Grün Optik, Wetzlar. The Zeeman splitting was accomplished by inserting specially designed gas discharge lamps between the poles of a strong permanent magnet. The graphite furnace was longitudinally heated and the samples were introduced into the tube manually by a pair of tweezers. Later on a solid sampler was developed, consisting of an automatic weighing and sample introduction system. This helped to speed up and standardise the analysis even more. The weighing of samples was accomplished by high precision micro-balances, such as Sartorius MP6 (sensitivity: 0.1μg).

The Grün Company did not succeed in keeping the pace with the rapid development on the AAS market especially for user friendly designed software. Although the Grün ZAAS instruments provided a great deal of important analytical information, today they are out of production.

Meanwhile another German firm, Analytik Jena, is providing an alternative AAS instrument with a D_2 background correction at a pulsation of 150 Hz. The instrument is equipped with a

transversally heated graphite tube of smaller dimension than the Grün tubes. A six-position lamp changer as well as many other options is operationally triggered from a state-of-the-art PC software. The sample introduction is carried out by a modified mechanical system. The solid sampler, originally designed for the Grün instrument is also available now and hence 30 to 40 individual analyses can be carried out per hour [19].

For INAA a standard setup consisting of a well shielded HPGe-detector (1,8 keV resolution, 25% rel. efficiency), Ortec amplifier and mutichannel analyzer was used. Maestro software for evaluation of gamma spectra and a home made programme for calculation of concentrations relative to synthetic multielement standards was used. Six aliquots of about 1.5 mg sample weight were irradiated at the FRJ-2 reactor in Jülich for 20 H at a neutron flux of 10^{14} N cm^{-2} s^{-1}. Three repetitive measurements after different cooling times were performed to obtain the results based on standard comparison with SRM 1547 (peach leaves). By recommendation of the producer this reference material is originally used at high sample masses (>250 mg). In this study, however, the material turned out to be sufficiently homogeneous to be used as a solid calibrant in a mass range of 0.1–2 mg.

Method for Homogeneity Characterisation:

Using an AAS instrument with D_2 background correction, prior to analysis a method has to be developed and optimised with respect to the temperature program and timing. Good peak shape is obtained only if most of the matrix is destroyed during the ashing step and if the analyte is effectively atomised within the shortest possible time (2-4 sec). Once the temperature program leads to satisfying peak shapes for a given material a calibration for the element of interest has to be performed. Calibrations using liquid standards or solid certified reference materials (CRMs) are possible. As AAS generally is rather matrix sensitive it is advisable to use well-characterised CRMs of similar matrix composition as the unknown sample for calibration. Increasing the sample mass introduced for analysis successively yields enough data points for calibration to cover the linear range for analysis. Checking the calibration can be performed by the analysis of a second CRM or any in-house reference material matching the matrix criteria. Finally this method together with the particular calibration is used for repetitive quantitative analyses to study the homogeneity of the materials. The scattering of individual results depends on the heterogeneity of the material plus the precision of the instrument for the particular element. The repeatability of the measurement process (precision) can be investigated for the elements considered by a series of liquid standard measurements in the relevant concentration range. It has to be verified that the precision of the instrument is small compared to the fluctuation of results caused by real sample measurements.

To quantify the homogeneity of an element in a given matrix a relative homogeneity factor was introduced by Kurfürst et al. based on a modification of Ingamells sampling constant [20,21].

Sampling Constant (Ingamells)**:**

$$K_s = RSD^2 \bullet m \qquad (1)$$

K_S = sampling constant
RSD = relative standard deviation
m = mean sample mass [mg]

Relative Homogeneity Factor (Kurfürst)**:**

$$H_E = RSD \bullet \sqrt{m} \qquad (2)$$

H_E = relative homogeneity factor
RSD = relative standard deviation *(of all measurements)*
m = mean sample mass [mg]

Homogeneity and minimum sample mass:

Using the instrument with the D_2 background correction, Cd and Pb were determined in IAEA-338 (lichen). Instrumental Neutron Activation Analysis (INAA) was applied to the same material for the determination of Na, K, Ca, Sc, Cr, Mn, Fe, Co, Zn, As, Br, Rb, Sb, Cs, Ba, La, Ce, Sm, Eu, Hf, W, Au, and Hg. For the calculation of homogeneity the H_E factor of Kurfürst seemed to be more suitable for small sample masses because of its quadratic relation rather than the linear sampling constant of Ingamells. In Figure 1 the relative standard deviation is plotted versus the sample mass.

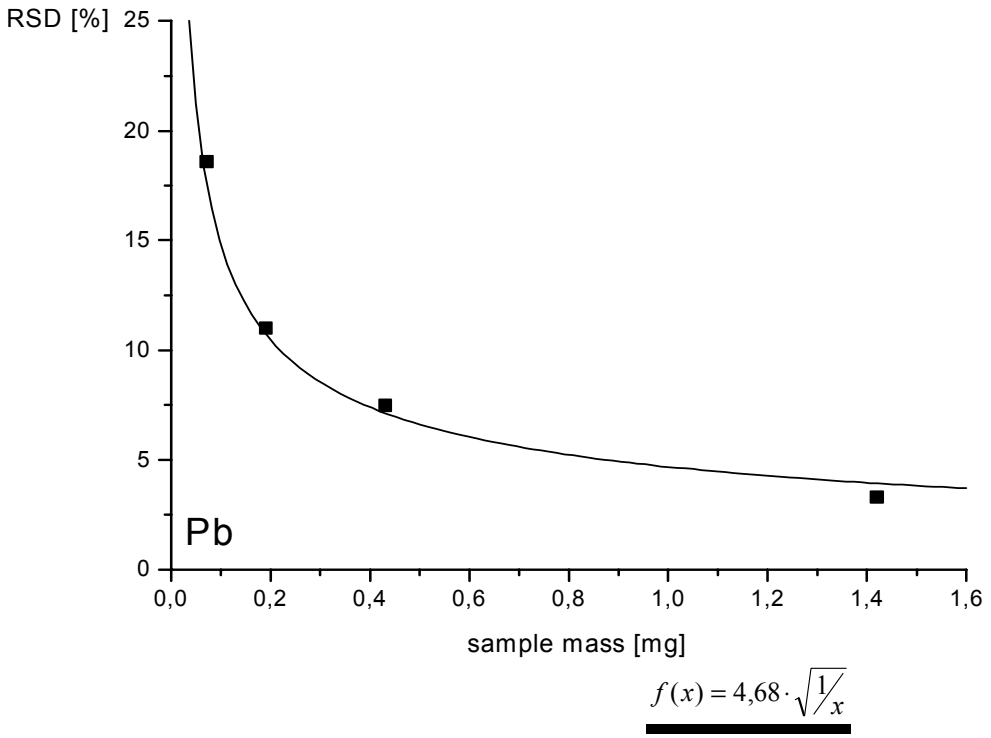

FIG. 1: *Relative Standard Deviation (RSD) [%] over sample mass [mg] for Pb in IAEA 393, Algae. Each data point represents at least 20 individual measurements [22].*

The smaller the sample mass the higher is the standard deviation of repetitive measurements and at masses greater than 2 mg the overall standard deviation levels off at an approximate value of the instruments precision (≅ 3%). The constant factor in the fitting curve (4.68) represents the homogeneity factor H_E of Pb in this particular material (IAEA 393, single cell algae) [23]. Now it seems to be feasible to calculate the standard deviation — which is now merely related to the material's homogeneity and not influenced by any methodological bias — according to equation (2) and report mean values according to expression (3).

$$\text{Concentration} = \text{Mean} \pm H_E\, m^{-1/2} \qquad (3)\ [24]$$

As the uncertainty of the mean is now related to the mass used for analysis and it is based on a careful evaluation of the element distribution in the material this approach could help to evaluate further the total uncertainty budget for various analytical techniques as it is recommended in accreditation and certification campaigns.

A statistical tolerance interval for 1mg sample mass according to equation (4) can be calculated. A minimum sample mass to achieve 5% precision on a 95% confidence level can be given by equation (5) [25] using a factor k'_2 for two-sided tolerance limits of normal distributions as given in standard statistical textbooks [26].

$$\Delta\ 1\ mg = k'_2 \bullet H_E \qquad (4)$$

$$M_{(5\%)} = (k'_2 \bullet H_E / UNC)^2\ m \qquad (5)$$

UNC denotes the uncertainty level at which M should be given (in our case ± 5%).

As an example we have calculated the minimum sample mass to obtain a 5% relative standard deviation in repetitive measurements for Pb in IAEA-338, lichen:

- distribution of results: normal
- uncertainty wanted: 5%
- k'_2 (p = 0.95, 1-α = 0.95, n = 100): 2.2313
- RSD of the homogeneity study: 9.5%
- mass used for homogeneity study: 0.211 mg

According to equation (5)

$$M = (2.2313 \bullet 9.5\%/ 5\%)^2 \bullet 0.211\ mg = 3.81\ mg$$

Most of the conventional analytical techniques available today cannot perform analyses on such a low sample mass and therefore — as the total analytical uncertainty seems not to be influenced by the material's heterogeneity — it is possible to quantify bias related to various steps (weighing, digestion, pipetting, diluting and measurement) in the analytical process of these materials.

Results and Discussion:

Repeatability of the SS-AAS instrument was checked using liquid standards prior to homogeneity studies. 40 measurements of 5 µl of a 0.1 ng/ml standard solution gave a mean of 0.0957 ± 0.004 ng/ml. From the 4.2% RSD about 1% can be allocated to the repeatability of the pipetting, hence less than 3.5% instrument stability for the measurement of Pb at this low concentration can be estimated.

For INAA the repeatability of the method was estimated from the relative standard deviation of six independent measurements of the same aliquot positioned each time on top of the HPGe detector. The results for some elements are given in Table 1.

In Tables 2 and 3 the SS-AAS results for homogeneity determination for Pb and Cd and minimum sample mass $M_{5\%}$ are given.

In Table 4 the results from INAA investigation for homogeneity of elements in IAEA-338 (lichen) are given:

TABLE I. REPEATABILITY OF 6 INDEPENDENT MEASUREMENTS OF ONE ALIQUOT SRM 1547 (PEACH LEAVES), 10000S EACH, 5 CM FROM THE DETECTOR

Element	Energy [keV]	peak statistics	mean [µg/g]	± SD	rel. SD [%]
Sm	103.	0.35	0.982	0.0218	2.2
Ce	145.	1.7	10.4	0.488	4,7
Cr	320.	15.1	0.941	0.170	18.1
La	328.	0.5	8.24	0.165	2.0
La	486.	0.34	8.46	0.098	1.2
La	1596.	0.33	8.35	0.167	1.99
Br	554.	0.81	10.6	0.367	3.46
Br	776.	0.69	10.4	0.297	2.85
Sc	889.	5.5	0.0437	0.00304	6.9
Rb	1077.	6.7	17.2	0.432	2.5
Fe	1099.	10.6	208.	11.9	5.7
Ca	1296.	3.9	15600.	1100.	6.9

TABLE II. HOMOGENEITY DETERMINATION OF PB IN IAEA CANDIDATE REFERENCE MATERIAL LICHEN BY SS-AAS

Bottle No.	weight [mg]	mean [µg/g]	± S	rel. SD [%]	n	H [% √m]	M [mg]
041	0.191	108.2	8.68	8.02	25	3.5	2.51
121	0.222	98.0	7.81	7.97	25	3.25	3.85
161	0.222	106.4	8.32	7.82	25	3.69	3.71
191	0.211	106.0	6.12	5.77	25	2.65	1.92
mean	0.211	103.9	9.89	9.52	100	4.54	3.81

TABLE III. HOMOGENEITY DETERMINATION OF CD IN IAEA 338, CANDIDATE REFERENCE MATERIAL LICHEN BY SS-AAS

Bottle No.	weight [mg]	mean [µg/g]	± SD	RSD [%]	n	H_{Cd}	M [mg]
012	0.339	0.447	0.0517	11.6	20	6.7	13.5
041	0.315	0.457	0.044	9.72	20	5.45	8.8
081	0.342	0.455	0.042	9.14	20	5.34	8.5
121	0.342	0.468	0.0373	7.97	20	4.66	6.45
161	0.335	0.426	0.033	7.82	20	4.52	6.1
191	0.343	0.45	0.043	9.6	20	5.62	9.4
mean	0.336	0.451	0.0418	9.3	120	5.37	5.6

TABLE IV. HOMOGENEITY FACTORS, MINIMUM SAMPLE MASS $M_{5\%}$ AND K'_2 FACTORS FOR ELEMENTS IN IAEA-338 (LICHEN) DETERMINED BY INAA

Element	mean [µg/g]	± SD	rel. SD [%]	n	H [%√m]	$M_{5\%}$ [mg]	k'_2-factor
Na	101.6	23.5	23.1	18	28.6	254.	2.7873
K	3044.	220.	7.2	18	8.96	24.6	2.7873
Ca	3990.	697	17.5	18	21.6	145.6	2.7873
Sc	0.212	0.0235	11.1	18	13.7	58.6	2.7873
Cr	4.96	0.45	9.12	9	11.3	58.7	3.3959
Mn	51.7	3.9	7.56	9	9.36	40.3	3.3959
Fe	852.	143.	16.8	18	20.8	134.	2.7873
Co	0.485	0.178	36.7	36	45.4	503.	2.4713
Zn	123.6	35.1	28.4	18	35.2	385.	2.7873
As	0.984	0.336	34.2	9	42.3	825.5	3.3959
Br	18.	1.46	8.1	35	10.0	24.7	2.4810
Rb	20.3	0.93	4.56	9	5.65	14.7	3.3959
Sb	0.502	0.055	11.0	18	13.7	57.5	2.7873
Cs	0.0837	0.0058	6.9	18	8.54	22.6	2.7873
Ba	27.4	2.18	7.97	9	7.1	44.8	3.3959
La	0.994	0.377	37.9	27	46.9	585.	2.5802
Ce	2.14	1.06	49.8	18	61.6	1180.	2.7873
Sm	0.124	0.016	12.9	9	16.0	117.	3.3959
Eu	0.0263	0.0035	13.3	26	16.5	73.	2.5966
Hf	0.0943	0.0054	5.76	9	7.1	23.4	3.3959
W	1.4	0.193	13.8	18	17.1	90.5	2.7873
Au	0.0076	0.0048	63.6	9	78.7	2855.	3.3959
Hg	0.318	0.026	8.18	9	10.1	47.2	3.3959

As can be seen from tables 2-4 elements such as K, Mn, Br, Rb, Cd, Cs, Ba, Hf and Pb can be considered as very homogeneous in this RM as they tend to show H_E values ≤ 10. The minimum sample mass $M_{5\%}$ was calculated for these elements ranging from 3.5 to 50 mg. Other elements such as Co, As, La, Ce, and Au tend to be rather unevenly distributed in this matrix (H_E values ≥ 40), hence minimum sample masses $M_{5\%}$ of $>$ 500 mg were calculated. Certification of these elements should be seriously discussed as the homogeneity seems not to be adequate in this material.

Conclusions:

Besides many other valuable applications of direct solid sample analysis homogeneity testing of solid materials seems to be a unique domain. INAA can also be applied for this purpose and can serve as a reference method in homogeneity studies. Valuable information on the material's properties can be gained and indispensable characteristic values can be calculated from these results. Particularly for the production and more efficient use of valuable CRMs the accurate homogeneity characterisation for a number of relevant analytes is mandatory and should be implemented from the reference material producers and reported in their certificates as well.

The common argument, that inconsistencies in repetitive measurements are automatically due to the materials heterogeneity seems not to be valid because heterogeneity in most of the investigated cases so far is visible only at the low mg sample mass range. Therefore the observed inconsistencies in most cases seem to be rather due to other analytical errors.

The availability of commercial instruments, user friendly software and wide experience makes direct solid sample analysis an attractive analytical method, particularly in combination with multi-element detection probabilities. Further developments will help to improve the calibration technique, the power of determination [27], the extraction of information from each analysis on more than one element, the speed of analysis by full automatisation and the versatility by development of convenient, robust and portable instruments for direct field analysis.

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A study on homogeneity of the IAEA candidate reference materials for microanalysis and analytical support in the certification of these materials

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Abstract. In this paper a study on homogeneity of new IAEA candidate reference materials: IAEA 338 Lichen and IAEA 413 Algae in small (ca.10 mg) samples as well as some data contributing to certification of these materials are presented.

1. INTRODUCTION

In the previous report [1] certification of a new biological reference material Virginia Tobacco Leaves (CTA-VTL-2) and the study on homogeneity of this and some other candidate reference materials was described.

As it is known, natural matrix certified reference materials (CRMs) are usually inhomogeneous on a microscopical scale and apparent homogeneity is achieved by grinding, sieving and mixing of a material so that a sufficiently great number of individual particles is present in a subsample taken for analysis. CRMs currently available from various producers have usually homogeneity guaranteed for sample weights of 100-250 mg [2-5] and sometimes even as high as 0.5-1.0 g [6-7]. This does not necessarily mean that smaller subsamples of these materials will show distinct inhomogeneity, but as the relevant information is not available they should not, in principle, be used below the sample mass recommended by the manufacturer.

On the other hand some microanalytical techniques such as energy dispersive X ray fluorescence (EDXRF), particle induced X ray emission (PIXE), solid sampling atomic absorption spectrometry (SS-AAS) use in fact smaller sample masses than those mentioned above and in this case no CRMs are practicaly available. Other techniques like e.g. instrumental neutron activation analysis (INAA) also have capability to use smaller sample masses than 100 mg. Therefore the search for CRMs which would be suitable for microanalytical techniques is of vital importance [8].

2. EXPERIMENTAL

Sample preparation

Ca. 10 mg amounts of IAEA 338 Lichen and IAEA 413 Algae were accurately weighed with the aid of analytical semi-micro balance (Precisa, Switzerland) into high purity polyethelene (PE) snap-cap capsules, 0.22 cm^3 (Faculteit der Biologie, Universiteit, Amsterdam). To avoid any contamination distribution of the samples into capsules was carried out using laminar air flow cabinet with HEPA-filter, Holten (Denmark).

Water content of the candidate RMs was determined in separate subsamples by drying for 24 h at 85^0 C.
Standard preparation

Stock solutions of Ce, Co, Cr, K, La, Sc, Sm, and Zn were prepared from metals, oxides or salts of spectral purity by dissolving in high purity acids, and diluting with 18 MOhm·cm water obtained from

Milli-Q RG ultra pure water system (Millipore Co.). Concentrations of stock solutions were usually close to 1mg/g of solution, sometimes 10mg/g of solution.

Working standard solutions were made from stock solutions by diluting with 18 MOhm·cm water. Concentration of the standard solutions were determined by weighing.

Multielement standards for INAA were prepared by transferring appropriate masses of standard solutions onto filter paper discs (ϕ = 7.7 mm) placed in analogous PE capsules as those used for the samples. After drying all capsules were closed and wrapped into Al foil similarly as the samples.

The amounts of elements in the standards were as follows: Standard I: Sm (0.25 μg), La (5 μg), Zn (530 μg), Co (13.5 μg) and Standard II: Ce (10 μg), Cr (45μg), K (215μg), Sc (0.6 μg).

Neutron activation analysis

The irradiation package consisting of 12 samples, 12 Zn flux monitors (2 mg each), and two multielement standards was irradiated for 3 hours in the reactor MARIA in Świerk at a thermal neutron flux of $1.6 \bullet 10^{13}$n cm^{-2} s^{-1} and cooled for about 16 hours.

γ ray spectrometric measurements were done with the aid of well type HPGe detector (CANBERRA) 180 cm^3 nominal volume, 30% relative efficiency, well depth 40 mm, well diameter 16 mm, resolution 2.3 keV for 1332.5 keV ^{60}Co line, coupled *via* ORTEC analog line to the multichannel analyzer TUKAN in the form of an ISA card inserted into a typical PC.

Good and reproducible geometry of measurements was assured by placing the samples in the flat-bottomed test tube at the bottom of the well. The results were corrected for the background. Blank (from the irradiation capsule) was usually negligible for most of the elements studied in this work, except for Cr.

Several measurements were performed in a live time mode after decay time of approximately: 16 h, 2 d, 7 d and 1-2 months after irradiation and the time of measurement varied from 500 to 100000 s.

Particle size measurements

Microscopic examination revealed that particles of algae have strong tendency to aggregate. To minimize this effect and obtain samples suitable for microscopic particle size measurements, the suspension of the material in water was agitated in an ultrasonic bath for 15 mins. then a drop was transferred onto the glass plate and dried in the air. Martin's diameters (arithmetic mean of the distance between opposite sides of a particle measured crosswise [9]) of 100-200 particles chosen at random were measured, using the microscope with x800 magnification.

The reliability of particle size measurements was confirmed by photographs obtained by standard procedure with the aid of scanning electron microscope DSM 942 .

3. RESULTS AND DISCUSSION

3.1. Methods of checking homogeneity

Two approaches were used for studying homogeneity of the IAEA's candidate CRMs. In the first, several samples taken from different containers were analyzed by INAA and the results compared with analogous results for several subsamples taken from single container. The two series of results were evaluated statistically comparing the variances by F-test and the means by t-test. Most of photopeaks with good or at least satisfactory counting statistics were utilized. For those elements for

which no standards were available, count rates normalized for a given sample mass and corrected for decay and decay during measurement were employed.

In the case of Lichen 338, the criterion $F < F_{0.05}$ is fulfilled for Br, Ce, Co, Fe, Na, Sc and Sm. The critical value ($F_{0.05}$) was slightly exceeded in the case of 487 keV photopeak of La. It should be also mentioned that measurable quantity of iridium was found to be present in a sample from one of the Lichen containers, while it was absent in all other containers. So, at least with respect to some elements, Lichen 338 shows some heterogeneity.

In the case of Algae 413 the criterion $F < F_{0.05}$ is fulfilled for As, Co, Cr, Fe, K and Na. The critical value $F_{0.05}$ is largely exceeded for Hg. In this case dispersion of results for samples taken from one container was greater than that for samples taken from various containers, suggesting that some local contamination might have ocurred during preparation, dispensing or packing of the material.

In the second approach, an attempt was made to calculate Ingamells' sampling constants as a quantitative measure of homogeneity (or inhomogeneity) of the samples under investigation.

The overall relative standard deviation (in percent): $R_o = (s/\bar{x})\cdot 100$ as determined from a series of replicate samples of approximately equal masses is composed of analytical error R_a and an error due to sample inhomogeneity R_s.

As the variances are additive one can write:

$$R_s^2 = R_o^2 - R_a^2 \qquad (1)$$

$$R_s = \sqrt{R_o^2 - R_a^2} \qquad (1a)$$

Analytical variance is in turn composed of several components and in our case these are: counting statistics R_c, neutron flux inhomogeneity $R_{fi,}$ irreproducibility of counting geometry $R_{g,}$ and weighing R_w. So, if the separate components of analytical variance will be determined, the R_a can be obtained from the relation:

$$R_a^2 = R_c^2 + R_{fi}^2 + R_g^2 + R_w^2 \qquad (2)$$

and then the sampling variance, R_s^2 and the error due to sample inhomogeneity R_s, can be derived from eq.(1). and (1a) respectively.

Ingamells [10,11] introduced into analytical vocabulary the term "sampling constant" K_s defined as:

$$K_s = R_s^2 \cdot m \qquad (3)$$

where: R_s^2 is sampling variance and m is sample mass.

K_s is expressed in the units of mass and is numerically equal to the sample mass necessary to limit the error due to sample inhomogeneity (sampling uncertainty) to 1% (with 68% confidence).

Some workers have been using also $K_s^{1/2}$ for characterizing homogeneity of materials [10,12].

In order to determine sampling variance accurately, it is necessary to minimize as much as possible the individual components of analytical variance. In this study, owing to the use of several Zn flux monitors

in the irradiation package, it was possible to correct the activities of the individual samples and standards for neutron flux inhomogeneity. The residual uncertainty due to flux inhomogeneity, R_{fi} was estimated to be lower than 1%. Counting statistics, R_c for most of the elements studied was in the range of 0.2-2%, and was substantially larger only for Ce, La and Co in Lichen and for Cd in Algae. Counting geometry, R_g was estimated from multiple measurements of the 559 keV photopeak of ^{76}As in the same sample, which after each measurement was removed and placed again in the detector well. The R_g thus determined amounted to 0.251%. The error of weighing R_w was assumed to be 0.1%.

The errors due to sample inhomogeneity (sampling uncertainties), R_s determined in this study for individual elements in IAEA 338 Lichen and IAEA 413 Algae as well as calculated sampling constants K_s are presented in Table 3.

3.2. Certified reference materials and microanalysis

The term "microanalysis" is not always unequivocally understood and used in literature and this remark applies also to the participants of the present CRP.

Two dictionary definitions of microanalysis will be quoted here: The first reads: "Ascertaining chemical composition from very small samples" [13], the second: "The analysis of quantities weighing 1 mg or less" [14]. While the first definition is of quite general character the problem arises what is "very small sample" remembering in addition that the meaning of such term, certainly may change with time. The second definition is more specific as it gives quantitative characteristics of the term which, however also can be challenged from various sides.

In INAA, analysis of samples weighing single milligrams is fully feasible [15], analysis of smaller samples is also possible but usually impractical, except of special cases e.g. short segments of human hair [16], or fragments of paint from art objects [17].

Solid sampling-Zeeman Atomic Absorption Spectrometry (SS-ZAAS) employs sample masses in the range: 0.02-20 mg for electrothermal atomization in a graphite furnace[18].

In micro-PIXE typical masses being analyzed are between 0.15 μg and 10 μg [19]. In X ray electron probe microanalysis the analyzed samples are even smaller (0.5-50 ng) [20]. All authors of the above mentioned papers describe their work as "**microanalysis"**. No doubt that the nomenclature in this domain needs much more precise definitions.

Coming back to our results one may note that in the case of Lichen, as shown in Table 3, sampling constants are mostly in the range of several tens or hundreds of milligrams. If the relation (3) reflects the real situation, then after the sampling constant for an analyte in a given material was determined, R_s values for different sample masses can be calculated. Such values for different elements in 338 Lichen are shown in Fig.3. One can easily note that for elements with $K_s \leq 0.100$ g, the R_s for 0.1 mg sample does not exceed 33% (*cf.* the data for Sc for which $K_s = 111$ mg), while for elements with $K_s = 2.8$ g, R_s for 0.1 mg sample will be already 168% (*cf.* data for La).

Similar plots for 413 Algae are shown in Fig. 4. Here for most of elements studied, K_s values are of the order of tens or even single milligrams. So, e.g. the expected R_s for 0.1 mg sample for Na is 5.3% ($K_s = 3.3$mg), that for Co 21% ($K_s = 44.4$mg), but the analogous value for Hg would be already 181, ($K_s = 3.3$g).

Homogeneity of powder materials should strongly depend on particle size. The number of spherical particles n in the sample of mass m is given by equation:

$$n = \frac{3\ m}{4\ \pi\ r^3\ d} \qquad (4)$$

where: d is material density, and r is particle radius.

Assuming that the median of the Martin's diameter can be taken as a *quasi-spherical diameter* and that density of both materials is ca. 0.7 g/cm^3, the number of particles in 10 mg of Lichen can be estimated as *ca.*. $5.3 \cdot 10^7$, and those in 10 mg of Algae as *ca.* $2.7 \cdot 10^7$ respectively. The last material according to preliminary assumptions of the manufacturers should contain almost identical particles (cells). The numbers of particles in 10 mg samples of the both materials are very similar so the differences in sampling constants of the same elements in the two materials are obviously due to the fact that Lichen consists of particles of conceivably very much more different elemental composition than Algae. On the other hand, the existence of significant differences in numerical values of sampling constants for individual elements in Algae shows however, that the real observed case is rather far from the initial assumptions, according to which all particles would be intrinsically homogeneous.

3.3. Elemental concentrations in the candidate RMs

One of the aims of the present CRP was to help IAEA in establishing "recommended" or "certified" values for some elements in the candidate RMs. The results of quantitative determinations of selected elements in the two materials are shown in Table 4. The results are presented as: mean ± standard deviation, together with relative percent standard deviation (RSD,%). These data were calculated on the basis of all results i.e. both those from various containers and those from single container.

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INAA studies of sampling properties of some natural matrix materials for the development of small sample reference materials

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Abstract. Instrumental neutron activation analysis (INAA) procedures were optimized for the analysis of small (1 mg) solid samples. This process included sample handling as well as detailed evaluation of high rate counting techniques, also in conjunction with rapidly decaying sources. The procedures provided the necessary analysis environment for the determination of large numbers of samples with high reproducibility. Existing biological and environmental reference materials as well as materials considered for development as certified reference materials were investigated. The analytical data obtained with the INAA procedures were used to determine homogeneity values for selected elements in the various materials. Based on these values development of reference materials for small sample techniques can be considered.

Introduction

Numerous solid sampling techniques have found applications in biological and environmental studies.[1] A significant problem in the use of these techniques, however, is a general lack in suitable certified reference materials (CRMs) and Standard Reference Materials® (SRM®s). Not only is the diversity of reference materials limited and closely matched samples are not always available to test the matrix affect on a technique's accuracy, but essentially no CRMs are certified for the small sample sizes that are typically used with solid sampling techniques.[2] Direct utilization of most existing CRMs in direct small sample analysis procedures, i.e. analyses of samples having masses considerably smaller than 100 mg, more typically 1 mg, is often difficult or even impossible because trace components may not be sufficiently homogeneously distributed in the sample or their homogeneous distribution has not been tested.

Investigations by INAA are used to explore the utility of existing CRMs and SRM®s for solid sampling techniques. The sampling characteristics are determined of some CRMs and other materials considered for development to specifically address their use with solid sampling techniques. The investigations focus on the use of statistical models, i.e. the Ingamells sampling constant[3], and the Kurfürst homogeneity factor[4], which describe chemical homogeneity of mixed component natural materials. INAA has the required characteristics for the determination of the input parameters to those models: practical absence of blank, good detection limits with respect to many elements, multielement capability, good penetration of neutrons through the matter, small absorption of gamma rays in the analyzed sample and good knowledge of potential sources of error. It is well suited for checking homogeneity with small and completely evaluated components of uncertainty in a given matrix and with sufficient sensitivity for the analysis of small samples. In this work, INAA procedures have been optimized for the homogeneity determinations of small (1 mg) solid samples.

Experimental

The objective of the implemented experimental approach has been the minimization and control of uncertainties in the analytical processes in order to facilitate the evaluation of uncertainties due to sample inhomogeneities. These processes included sample handling as well as the INAA procedures including high rate counting techniques, also in conjunction with rapidly decaying sources, to establish the necessary analysis environment for the determination of large numbers of samples with high reproducibility.

Sample Preparation

The investigated natural matrices included biological materials such as peach leaves (SRM® 1547), lichen (IAEA 338, prepared by the IAEA Laboratories Seibersdorf, Austria), and bovine liver (IAEA-386, prepared by the Shanghai Institute of Nuclear Research, Shanghai, China), as well as environmental materials such as urban particulate matter (SRM®s 1648 and 1649) and ocean sediments (SED-M, deep ocean sediment[5] and SED-K, antarctic sediment provided by Kniewald) . The biological materials had average particle sizes of <10 μm to 20 μm;[6] the urban particulate matter 30 μm; the sediments 8 μm and 20 μm respectively. The materials were prepared for INAA by pressing them into 0.5 mm to 1 mm thick, 13 mm diameter tablets from which small subsamples were taken with a 1.5 mm diameter corer. These mini-tablets had a mass of 0.5 mg to 3 mg depending on the matrix and thickness of the initial tablet. The small sample tablets were transferred to an aluminum weighing pan and weighed on a micro balance (Mod. UMT2, Mettler Toledo, Inc., Hightstown, NJ) . The tablets were then sealed in 0.5 cm^2 bags made from polypropylene film (Spectro Film™ Polypro-Econo 6.3, Somar International, Inc., Tuckahoe, NY) for irradiation. Since quantitative transfer after irradiation was deemed impossible and the radioactive small samples could not be re-weighed, this specific thin film polypropylene was selected as the best suitable container material. This material does not contain significant amounts of trace elements that must be considered for blank corrections, except for sodium and chlorine at the tens of ppm level which had to be taken into account for some of the investigated materials with similar sodium and chlorine content. The use of pellets and their transfer for weighing resulted in more accurate sample masses then direct weighing of the materials in the bags because of the reduction in static charge effects.

INAA Procedure

The INAA procedure followed the established principles at the NIST Nuclear Methods Group.[7] For irradiation, the sample bags were enclosed in pre-cleaned secondary polyethylene bags bearing the sample ID numbers. These were then placed in fixed positions in the irradiation containers together with pipetted and dried multi-element standard solutions (10 μL or 20 μL on 5 mm diameter filter paper), either individually with nickel or zinc flux monitors, or in groups. Irradiations were carried out in the RT-4 pneumatic irradiation facility of the NIST research reactor at a neutron fluence rate[8] of $3.5 \cdot 10^{17}$ $m^{-2}s^{-1}$ for 12 s to 120 s and 8 h, respectively. After irradiation and appropriate decay, the secondary containers were removed and the samples were counted. Commonly one count for 600s was used to assay short-lived nuclides (after the short irradiations). For the assay of longer lived nuclides (after the long irradiations) a first count of 0.5 h to 3 h after 3 d to 7 d decay and a second count of 12 h after more than 14 d decay was used. The associated flux monitors were generally counted to acquire 500000 counts in the indicator peak areas.

To minimize the inherent counting uncertainty, these experiments mainly utilized a newly installed set of three high resolution gamma ray detectors (Gamma gauge, EG&G Ortec, Oak Ridge, TN, in the range of 30% relative efficiency and 1.7 keV to 1.8 keV resolution); two detectors with high count rate capabilities through transistor reset preamplifiers.[9] Each detector is operated with a gaussian amplifier at 3 μs shaping time (Mod. 672, EG&G Ortec) to preserve detector resolution, a 6 μs fixed conversion time ADC with 16k channel combined with a loss-free counting module, and an ethernet acquisition interface module (581 ADC, 599 LFC, 556 AIM, Canberra Industries, Meriden, CN), controlled by the Canberra Genie systems spectroscopy and applications software based on a DEC 3000 workstation computer under VMS (Digital Equipment Corp., Maynard, MS). Count rates in short and intermediate halflife assays reached up to 30000 s^{-1}. The three detectors provided efficiency in sample throughput and allowed for the simultaneous count of three samples or standards from the same irradiation, this was used for the assay of short and intermediate half life nuclides. The long counts were carried out on a conventional detector system equipped with a sample changer. All data evaluations were done with the peak search and activation analysis software from Canberra.

Results and Discussion

Uncertainty Budget

The results of activation analysis measurements are subject to well-known common analytical sources of uncertainties as well as method specific uncertainties as, e.g. summarized by Greenberg.[10] For these INAA experiments that werer intended to measure differences in induced activity, i.e. differences due to inhomogeneity in the amount of analyte in a given test portion, the experimental procedure was designed to allow only the following uncertainties to be part of the result:
uncertainty due to inhomogeneity u_{HOM},
uncertainty due to counting statistics u_c,
uncertainty due to activation u_{irr}
uncertainty due to the gamma spectrometric measurement u_m.
Uncertainties relating to the determination of accurate quantitative results were not relevant in these experiments. The observed experimental variance of the INAA results was a summation of the variances of homogeneity and the relevant analytical components (u_{AN}) as shown in eq. 1:

$$u_{exp}^2 = u_{HOM}^2 + u_c^2 + u_{irr}^2 + u_m^2 \qquad (u_{irr}^2 + u_m^2 = u_{AN}^2) \quad (1)$$

Knowing the listed analytical components of variance allows the determination of the homogeneity component.

The determination and control of uncertainty due to counting statistics (u_c) is rather straightforward; this uncertainty is largely dependent on the sample composition, the decay characteristics of the indicator nuclides, and the assay parameters. The applied procedure optimized irradiation, decay, and counting parameters to maximize peak to background ratios in order to obtain counting statistical uncertainties in a desirable range of 1% to 0.1% relative for the majority of analytes assayed. This required peak areas of tens to hundreds of thousand counts. In the case of rapidly decaying activities this was essentially achieved with high count rate capabilities of the gamma spectrometers. The control of the irradiation uncertainties at the NIST reactor irradiation facilities has been discussed earlier;[11] and the data have been verified in this work. Depending on irradiation time and mode, i.e. fixed rabbit position or 180 degree inverted irradiations, u_{irr} ranged from 0.2% to 0.08% relative. The uncertainty due to the gamma spectrometry measurement (u_m) is a combination of uncertainties in measurement geometry, spectrometry system data throughput and gamma spectrum evaluation. The measurement geometry for small sample analyses approaches almost ideal conditions since essentially point sources are produced in the INAA procedure. This uncertainty is commonly estimated at several tenths of a percent, but has been negligible in these experiments due to the geometrical similarity of the samples. The dependency of the gamma spectrometry measurements from the count rates has been checked by evaluating spectra obtained with the LFC system in dual counting mode, i.e. accumulating simultaneously the live spectral data and the corrected data. No increase beyond corrected counting statistics due to the loss-free counting technique was observed in the measurement uncertainty, whereas the application of standard pile-up corrections to the same data was affected by uncertainties in the pile-up correction factor.[12] Uncertainties due to the gamma spectrometry evaluation process can be held small when the spectrum shape is the same for all measurements. Under these conditions, the relative uncertainties due to the measurement ($u_{m)}$ were estimated in the range of 0.5% to 0.3%.

Determination of homogeneity

The observed elemental variances of each measurement experiment and the discussed components of the analytical variances are used to calculate variances due to inhomogeneity for each element that are converted to relative uncertainties (R) for input into the two relevant equations that are commonly used to express elemental homogeneity of a sample as a function of sample mass (w):

Ingamells sampling constant K_s

$$K_s = R^2 * w \quad (2)$$

Kurfürst elemental homogeneity factor H_e

$$H_e = R_{HOM} * \sqrt{w} \quad (3)$$

Equation 2 allows to calculate the sample mass (here in mg) required to not exceed a certain relative uncertainty due to heterogeneity for the investigated analyte, K_s expressing the sample mass where 1% relative standard deviation would be determined for a set of normal distributed results. Equation 3 gives for H_e the relative standard deviation in percent for the element of interest if a 1 mg sample would be repeatedly analyzed and no analytical uncertainty would influence the result. The values from the latter approach appear to be less scattered than Ingamells' sampling constant. Kurfürst elemental homogeneity factors generally are regarded as acceptable for trace element analysis when they are below 10.

Homogeneity factors in test materials

The process for determining homogeneity values is exemplified with results from a candidate SRM® Abyssal Sediment. This sediment, collected from "Station M" in the Northern Pacific, should be suitable for small sample analysis due to the likelihood of homogeneous formation, its fine natural particle size (8 µm to 20 µm), and the satisfactory sampling data obtained in this work. Table 1 summarizes the analytical results and components of the uncertainty budget for 18 elements with elemental mass fractions ranging from several percent to the mg/kg level. The relative uncertainties in the study of 1.6 mg sample masses were as follows: u_{exp}= 0.9% to 11%, u_{AN}= 0.3% to 6.6%, u_{HOM}= 0.9% to 11%, with the resulting K_s= 1 mg to 180 mg and H_e = 1 to 13. The correctness for the estimated analytical uncertainties is confirmed with elements that are assayed with two gamma ray lines that give similar results. Caesium and zinc showed unsatisfactory results, u_{exp} for Zn is larger than expected due to difficulties in the gamma spectrometry evaluation of the 1115 keV line because of very large scandium peaks at 1120 keV. Alternate evaluation techniques do not reduce the additional uncertainty, however a much larger number of analyses could enable the proper identification of contributions to u_{exp}.

It must be noted that the analysis of the uncertainty budget gives somewhat ambiguous results for contributions from sampling inhomogeneities when u_{exp} is small, in these cases much smaller sample sizes should be taken. Similarly the homogeneity results appear more random when u_{exp} is rather large, e.g. due to the large u_c associated with the INAA assay for some elements with low INAA sensitivity, their homogeneity must be determined with a much larger number of experimental data points or with a different technique.

The described process has been used for a number of existing and candidate CRMs. Table 2 lists the Kurfürst elemental homogeneity factors for elements determined with INAA via short-lived nuclides. The results illustrate that existing CRMs cannot unconditionally be used with small sample procedures, but that extensive measurements may show for which elements small sample analysis may produce reliable results. The potential heterogeneity for Al in SRM 1547 was reported earlier by separating a silicate fraction from the material.[13] Sodium and chlorine are probably more homogeneous in SRM 1547 than indicated in Table 2 due to inhomogeneity of the subtracted blank contribution which was equal to the elements' mass fractions in this material.

Conclusions

Because of its dynamic range of elemental sensitivity, INAA is well suited to study the homogeneity of small samples. Its analytical uncertainties can be sufficiently controlled and can be easily determined to obtain from experimental data the contribution of material inhomogeneity to the uncertainty budget. Not unexpectedly, all the investigated finely dispersed matrices, whether they had been processed to a small uniform particle size, e.g. by air-jet milling, such as in peach leaves and lichen, or occurring naturally at a small particle size, such as in air particulate or deep ocean sediment, exhibited the desired homogeneity for many trace constituents. From these results it can be inferred that the investigated materials can be used with many of the solid sampling techniques. The SRM® program of NIST may make some existing natural matrix SRM®s available for use with small sample techniques after a comprehensive homogeneity evaluation.

TABLE I. HOMOGENEITY RESULTS FROM INAA MEASUREMENTS ON CANDIDATE SRM ABYSSAL SEDIMENT. THE MEASURED SAMPLE MASS WAS 1.6 MG. THE ELEMENTS ARE RANKED TOWARDS INCREASING HETEROGENEITY AS EXPRESSED IN THE CALCULATED UNCERTAINTY FOR HOMOGENEITY U_{HOM} FOR THIS SAMPLE SIZE, THE KURFÜRST HOMOGENEITY FACTOR H_E, OR THE INGAMELLS SAMPLING CONSTANT K_S

Element (γ in keV)	Mass fraction (mg/kg)	u_{exp} (%)	u_c (%)	u_{AN} (%)	u_{HOM} (%)	H_e	K_s (mg)
Al	65050	0.91	0.223	0.3	0.830	1.05	1.10
V	147.6	1.63	1.229	0.3	1.028	1.3	1.69
Fe (1099)	44623	1.45	0.522	0.5	1.257	1.59	2.53
Sc (889)	17.51	1.52	0.249	0.5	1.414	1.79	3.2
Fe (1292)	44626	1.69	0.526	0.5	1.526	1.93	3.73
Cl (2178)	36400	1.63	0.436	0.3	1.542	1.95	3.8
Mn (846)	993	1.62	0.233	0.3	1.575	1.99	3.97
Na (1368)	33900	1.69	0.463	0.3	1.597	2.02	4.08
Cl-1642	36500	1.78	0.521	0.3	1.675	2.12	4.49
Mn (1811)	984	2.43	0.783	0.6	2.221	2.81	7.89
Na (2754)	33500	2.40	0.483	0.3	2.332	2.95	8.70
Sc (1120)	17.14	2.40	0.205	0.5	2.338	2.96	8.75
Mg (843)	23930	4.54	2.24	0.5	3.917	4.95	24.6
Th	10.6	4.31	1.48	0.5	4.017	5.08	25.8
Mg-1014	23600	4.55	1.979	0.5	4.066	5.14	26.4
Ce	59.91	5.02	1.247	0.5	4.837	6.12	37.4
Cr	115.6	5.28	1.224	0.5	5.112	6.47	41.8
Ti	4000	6.86	4.173	0.5	5.422	6.86	47.0
Rb	110	8.93	6.592	0.5	6.004	7.59	57.7
Sm	6.78	8.89	5.766	0.5	6.748	8.54	72.9
Hf	3.28	8.68	4.848	0.5	7.183	9.09	82.6
Cs	6.47	10.44	3.637	0.5	9.773	12.4	152
Zn	296	11.01	2.54	0.5	10.70	13.5	183.

TABLE II**.** KURFÜRST ELEMENTAL HOMOGENEITY FACTORS FOR SELECTED RMS DETERMINED FROM EXPERIMENTAL UNCERTAINTIES WITH AN INAA PROCEDURE USING SHORT-LIVED INDICATOR NUCLIDES. THE SAMPLE MASSES RANGED FROM 0.5 MG TO 2.5 MG; THE NUMBER OF DETERMINATIONS WERE 12 FOR EACH MATERIAL

Material /Element	Al	Ca	Cl	Cu	Mg	Mn	Na	Ti	V
SRM® 1648 Urban Particulate Matter	0.10	5.5	3.1	-	3.5	0.84	1.2	0.86	0.52
SRM® 1649 Urban Dust/Organics	1.1	<5	5.4	-	2.6	2.0	4.2	<5	1.6
Candidate SRM® Abyssal Sediment (M)	1.0	-	1.5	-	4.1	1.6	1.6	5.4	1.3
Candidate SRM® Antarctic Sediment (K)	2.1	1.3	9.7	-	5.4	3.3	5.5	6.4	2.2
SRM® 1547 Peach Leaves	17.7	2.0	6.2	-	2.0	1.5	10.7	2.2	5.1
IAEA-338 Lichen	2.1	1.4	1.1	-	5.6	0.30	2.0	4.0	0.70
IAEA-386 Bovine Liver	-	-	3.4	2.9	1.7	8.9	2.5	-	-

Certain commercial equipment, instruments or materials are identified in this paper in order to specify the experimental procedures in adequate detail. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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ANNEXES

Annex 1

TABLES

TABLE I. RESULTS OF REPETITIVE ANALYSIS USING NUCLEAR ANALYTICAL METHODS IN IAEA-338 (LICHEN). R_0 = TOTAL VARIANCE, R_A = ANALYTICAL VARIANCE, R_S = VARIANCE DUE TO HETEROGENEITY. GREY MARKED VALUES EITHER DO NOT MATCH THE CRITERIA $R_0/R_A > 1$, OR WERE IDENTIFIED AS OUTLYERS.

Method	sample size		Element	No of observations	R_0	R_A	R_S	Ingamels K_S
NAA	120	mg	Ag	5	9.60	17.80	#NUM!	
NAA	10	mg	Al	10	2.84		2.84	80.7
NAA	120	mg	Al	5	2.33	1.42	1.84	406.3
NAA	1.5	mg	Al	12	1.80	0.59	1.70	4.34
SRXRF	0.1	µg	As	7	65.70		65.70	0.43
NAA	1.5	mg	As	9	34.15	5.50	33.70	1704
NAA	10	mg	As	10	12.57		12.57	1580
NAA	120	mg	As	5	8.55	2.70	8.12	7912
NAA	1.5	mg	Au	9	48.00	21.00	43.16	2794
NAA	3	mg	Au	15	36.21	16.83	32.06	3084
NAA	120	mg	Au	5	11.09	7.82	7.86	7413
NAA	1.5	mg	Ba	9	7.96	8.00	#NUM!	
NAA	120	mg	Ba	5	2.93	3.88	#NUM!	
SRXRF	0.1	µg	Br	7	34.50	1.70	34.46	0.119
NAA	1.5	mg	Br	35	8.11	3.50	7.32	80.3
SRXRF	0.1	µg	Br	9	6.90	1.70	6.69	0.0045
NAA	10	mg	Br	10	5.33		5.33	222
PIXE	0.6	mg	Br	7	4.80	2.00	4.36	11.4
NAA	3	mg	Br	15	3.16	1.10	2.96	26.3
NAA	120	mg	Br	5	2.29	1.00	2.06	1049
NAA	10	mg	Br (554)	6			3.31	109
NAA	10	mg	Br (776)	6			2.87	82.4
SRXRF	0.1	µg	Ca	7	33.10	2.00	33.04	0.109
NAA	1.5	mg	Ca	18	17.47	6.90	16.05	120
PIXE	0.6	mg	Ca	7	10.40	2.00	10.21	62.5
NAA	120	mg	Ca	5	9.30	3.46	8.63	893.7
SRXRF	0.1	µg	Ca	9	8.50	2.00	8.26	0.0068
NAA	10	mg	Ca	10	4.85		4.85	235
NAA	1.5	mg	Ca	12	3.50	3.31	1.13	1.9

µ PIXE	0.209	µg	Ca	12	3.36	0.96	3.22	0.0002
µ PIXE	5.01	µg	Ca	12	2.14	0.73	2.01	0.02
PIXE	1640	µg	Ca	9	1.53	0.52	1.44	3.4
EPXMA	490	ng	Ca	3074 particles			5.00	0.012
NAA	120	mg	Cd	5	20.24	26.00	#NUM!	
SS-AAS	0.336	mg	Cd	120	9.30	2.80	8.87	26.4
NAA	1.5	mg	Ce	18	49.53	4.70	49.31	3647
NAA	120	mg	Ce	5	2.18	1.86	1.13	153
NAA	10	mg	Ce	6			7.01	491
PIXE	0.6	mg	Cl	7	34.00	6.00	33.47	672
NAA	10	mg	Cl	10	5.15		5.15	265
NAA	120	mg	Cl	5	3.05	1.52	2.65	843
µ PIXE	0.164	µg	Cl	12	3.02	1.11	2.81	0.0013
NAA	3	mg	Cl	15	2.81	1.93	2.04	12.5
PIXE	1289	µg	Cl	9	2.30	0.89	2.12	5.79
µ PIXE	3.94	µg	Cl	12	1.71	0.86	1.48	0.0086
NAA	1.5	mg	Cl-1642	12	1.06	0.61	0.86	1.11
NAA	1.5	mg	Cl-2178	12	1.20	0.58	1.05	1.65
NAA	1.5	mg	Co	36	36.70	6.50	36.12	1957
NAA	120	mg	Co	5	2.06	1.42	1.49	266
NAA	10	mg	Co (1173)	6			6.10	372
NAA	10	mg	Co (1332)	6			6.56	430
SRXRF	0.1	µg	Cr	9	129.00	50.00	118.90	1.41
SRXRF	0.1	µg	Cr	7	87.80	50.00	72.17	0.52
PIXE	0.6	mg	Cr	7	30.00	25.00	16.58	165
NAA	1.5	mg	Cr	9	9.07	7.00	5.77	49.9
NAA	3	mg	Cr	15	6.72	5.64	3.65	40
NAA	120	mg	Cr	5	2.04	1.38	1.50	270
NAA	1.5	mg	Cs	18	6.65	5.86	3.14	14.8
NAA	120	mg	Cs	5	1.54	1.60	#NUM!	
SRXRF	0.1	µg	Cu	7	44.00	7.00	43.44	0.189
SRXRF	0.1	µg	Cu	9	8.10	7.00	4.08	0.0017
PIXE	0.6	mg	Cu	7	8.00	2.00	7.75	36
NAA	1.5	mg	Eu	26	13.31	4.30	12.59	238
NAA	120	mg	Eu	5	4.65	3.00	3.55	1512

SRXRF	0.1	µg	Fe	7	32.60	5.00	32.21	0.104
NAA	1.5	mg	Fe	18	16.78	5.70	15.79	374
µ PIXE	0.243	µg	Fe	12	6.60	3.46	5.62	0.0077
SRXRF	0.1	µg	Fe	9	5.50	5.00	2.29	0.0005
PIXE	0.6	mg	Fe	7	4.70	4.00	2.47	3.66
µ PIXE	5.83	µg	Fe	12	4.52	2.65	3.66	0.078
PIXE	1907	µg	Fe	9	2.31	2.20	0.69	0.91
NAA	120	mg	Fe	5	1.63		1.63	317.5
EPXMA	2500	ng	Fe	15683 particles			5.00	0.063
NAA	10	mg	Fe (1099)	6			1.77	31.3
NAA	10	mg	Fe (1291)	6			2.12	44.9
NAA	1.5	mg	Hf	9	5.73	2.80	5.00	37.5
NAA	120	mg	Hf	5	3.18	4.44	#NUM!	
NAA	10	mg	Hg	10	20.24		20.24	4096
NAA	1.5	mg	Hg	9	8.18	10.00	#NUM!	
NAA	120	mg	I	5	15.22	15.00	2.58	799
NAA	10	mg	I	10	10.17		10.17	1034
SRXRF	0.1	µg	K	7	33.80	5.00	33.43	0.112
PIXE	0.6	mg	K	7	20.60	4.00	20.21	245
NAA	1.5	mg	K	18	7.23	6.00	4.03	24.4
SRXRF	0.1	µg	K	9	6.90	5.00	4.75	0.0023
µ PIXE	4.74	µg	K	12	4.82	0.82	4.75	0.107
µ PIXE	0.197	µg	K	12	2.52	1.03	2.30	0.001
NAA	120	mg	K	5	2.32	2.50	#NUM!	
NAA	3	mg	K	15	1.87	1.39	1.25	4.69
NAA	10	mg	K	6			3.04	92.4
EPXMA	25	ng	K	158 particles			5.00	0.0006
NAA	120	mg	La	5	127.65	1.38	127.64	1.90E+06
NAA	1.5	mg	La	27	37.93	2.00	37.87	2151
NAA	10	mg	La (1596)	6			16.75	2806
NAA	10	mg	La (487)	6			11.43	1306
NAA	120	mg	Lu	5	17.57	6.74	16.23	31610
NAA	10	mg	Mg	10	19.11		19.11	3652
EPXMA	650	ng	Mg	4078 particles			5.00	0.0163
NAA	1.5	mg	Mg-1014	12	6.07	3.99	4.57	31.3

SRXRF	0.1	µg	Mn	7	28.40	3.00	28.2	0.0797
NAA	1.5	mg	Mn	9	7.54	6.10	4.44	29.6
SRXRF	0.1	µg	Mn	9	7.00	3.00	6.32	0.004
NAA	10	mg	Mn	10	6.15		6.15	378
PIXE	0.6	mg	Mn	7	6.00	2.50	5.45	17.8
NAA	120	mg	Mn	5	1.80	1.16	1.38	229
NAA	3	mg	Mn	5	2.01	1.15	1.65	8.2
NAA	10	mg	Mn	6			2.28	52
NAA	1.5	mg	Mn-1811	12	0.84	0.78	0.31	0.144
NAA	1.5	mg	Mn-846	12	0.63	0.58	0.24	0.086
SRXRF	0.1	µg	Mo	7	36.10	10.00	34.69	0.12
SRXRF	0.1	µg	Mo	9	19.20	10.00	16.39	0.027
NAA	120	mg	Mo	5	3.22	24.00	#NUM!	
NAA	1.5	mg	Na	18	23.15	15.00	17.63	466
NAA	10	mg	Na	10	3.86		3.86	149
NAA	120	mg	Na	5	1.89	1.28	1.39	232
NAA	3	mg	Na	15	1.31	1.11	0.70	1.47
NAA	10	mg	Na	6			2.68	71.8
NAA	1.5	mg	Na-1368	12	2.91	2.38	1.67	4.18
PIXE	0.6	mg	Ni	7	63.30		63.3	2404
NAA	120	mg	Ni	5	9.43	14.80	#NUM!	
EPXMA	22	ng	P	136 particles			5.00	0.0006
SRXRF	0.1	µg	Pb	7	32.60		32.60	0.106
PIXE	0.6	mg	Pb	7	9.80		9.80	57.6
SS-AAS	0.21	mg	Pb	100	9.52	3.20	8.97	16.9
SRXRF	0.1	µg	Pb	9	7.80		7.80	0.0061
SRXRF	0.1	µg	Rb	7	36.70	5.00	36.36	0.132
PIXE	0.6	mg	Rb	7	10.70	8.00	7.11	30.3
SRXRF	0.1	µg	Rb	9	9.10	5.00	7.60	0.0058
NAA	1.5	mg	Rb	9	4.58	2.50	3.84	22.1
NAA	120	mg	Rb	5	1.74	0.90	1.49	266
PIXE	0.6	mg	S	7	28.90	15.00	24.70	366
PIXE	1096	µg	S	9	4.07	1.39	3.83	16.1
EPXMA	21	ng	S	132 particles			5.00	0.0005
NAA	1.5	mg	Sb	18	10.96	2.80	10.59	168
NAA	3	mg	Sb	15	7.94	7.49	2.64	20.9
NAA	120	mg	Sb	5	3.04	1.70	2.52	762

NAA	1.5	mg	Sc	18	11.08	6.90	8.68	113
NAA	3	mg	Sc	15	2.41	1.91	1.47	648
NAA	120	mg	Sc	5	1.71	1.00	1.39	232
NAA	10	mg	Sc	6			3.33	111
NAA	10	mg	Se	10	21.09		21.09	4448
PIXE	484	µg	Si	9	7.55	1.51	7.40	2.65
µ PIXE	0.062	µg	Si	12	6.57	1.15	6.47	0.0026
µ PIXE	1.48 ug	µg	Si	12	5.13	0.85	5.06	0.038
NAA	1.5	mg	Sm	9	12.90	4.20	12.20	223
NAA	120	mg	Sm	5	5.51	1.58	5.28	3345
NAA	10	mg	Sm	6			2.70	72.9
PIXE	0.6	mg	Sr	7	31.30		31.30	588
NAA	120	mg	Ta	5	2.74	4.96	#NUM!	
NAA	120	mg	Tb	5	18.06	7.60	16.38	32196
NAA	3	mg	Th	15	7.59	8.85	#NUM!	
NAA	120	mg	Th	5	2.84	1.64	2.32	646
SRXRF	0.1	µg	Ti	9	48.70	15.00	46.33	0.215
SRXRF	0.1	µg	Ti	7	25.90	15.00	21.11	0.0446
NAA	120	mg	Ti	5	5.43	15.60	#NUM!	
PIXE	0.6	mg	Ti	7	5.40	5.00	2.04	2.5
NAA	1.5	mg	Ti	12	4.36	2.92	3.23	15.6
NAA	10	mg	V	10	7.79		7.79	607
NAA	120	mg	V	5	3.98	4.24	#NUM!	
NAA	1.5	mg	V	12	1.11	0.94	0.59	0.52
NAA	120	mg	W	5	16.16	14.00	8.07	7815
NAA	1.5	mg	W	18	13.79		13.79	285
NAA	120	mg	Yb	5	4.74	16.00	#NUM!	
SRXRF	0.1	µg	Zn	7	33.50	4.00	33.26	0.111
NAA	1.5	mg	Zn	18	28.40	4.70	28.01	1177
SRXRF	0.1	µg	Zn	9	12.00	4.00	11.31	0.013
PIXE	0.6	mg	Zn	7	3.90	2.00	3.35	6.73
NAA	3	mg	Zn	15	3.50	1.87	2.96	26.3
NAA	120	mg	Zn	5	1.91	1.00	1.62	315

TABLE II. RESULTS OF REPETITIVE ANALYSIS USING NUCLEAR ANALYTICAL METHODS IN IAEA-413 (SINGLE CELL ALGAE, ELEVATED LEVEL). R_0 = TOTAL VARIANCE, R_A = ANALYTICAL VARIANCE, R_S = VARIANCE DUE TO HETEROGENEITY. GREY MARKED VALUES EITHER DO NOT MATCH THE CRITERIA $R_0/R_A > 1$, OR IDENTIFIED AS OUTLYERS

Method	sample size [mg]	Element	No of observations	R_0	R_A	R_S	Ingamels K_S
NAA	120	Al	6	6.00	2.07	5.63	3807.51
NAA	10	Al	10	5.66		5.66	320.40
PIXE	0.6	As	6	7.40	1	7.33	32.26
SRXRF	0.0001	As	6	6.30	1	6.22	0.00
NAA	10	As	10	5.35	4.5	2.89	83.46
NAA	1.2	As	12	5.30	3.3	4.15	20.64
SRXRF	0.0001	As	9	5.10	1	5.00	0.00
NAA	120	As	6	1.34	0.5	1.25	187.01
NAA	100	As	15			1.16	134.56
NAA	10	As	6			1.31	17.16
NAA	1.2	Au	6	22.00	10	19.60	460.80
NAA	120	Ba	6	6.87	5.46	4.17	2088.33
SRXRF	0.0001	Br	9	44.70	15	42.11	0.18
SRXRF	0.0001	Br	6	24.70	15	19.62	0.04
PIXE	0.6	Br	6	14.00	2	13.86	115.20
NAA	1.2	Br	6	9.40	7.5	5.67	38.53
NAA	120	Br	6	3.30	1.78	2.78	926.38
NAA	100	Br	15			1.18	139.24
SRXRF	0.0001	Ca	6	12.20	2.5	11.94	0.01
NAA	10	Ca	10	7.06		7.06	499.08
PIXE	0.6	Ca	6	6.90	1.5	6.73	27.22
SRXRF	0.0001	Ca	9	6.80	2.5	6.32	0.00
NAA	120	Ca	6	6.00	4.77	3.64	1590.94
PIXE	1.64	Ca	6	2.85	1.6	2.36	9.12
Micro PIXE	0.005	Ca	12	1.61	0.78	1.41	0.01
Micro PIXE	0.00021	Ca	12	1.39	1.07	0.89	0.00
NAA	1.2	Cd	12	3.50	2.5	2.45	7.20
NAA	3	Cd	15	2.88	1.09	2.66	21.25
NAA	120	Cd	6	1.73	1	1.41	239.47
NAA	100	Cd	15			1.23	151.29
NAA	10	Cd/In	6			6.66	443.56

NAA	120	Cl	6	7.35	3.166	6.63	5272.31
NAA	10	Cl	10	4.41		4.41	194.53
NAA	1.2	Co	4	3.35	2	2.69	8.67
NAA	120	Co	6	2.33	1	2.10	529.44
NAA	100	Co	15			2.42	585.64
NAA	10	Co (1173)	6			2.10	44.10
NAA	10	Co (1332)	6			1.72	29.58
SRXRF	0.0001	Cr	6	10.00	1.7	9.85	0.01
SRXRF	0.0001	Cr	9	7.70	1.7	7.51	0.01
NAA	1.2	Cr	12	7.60	4.7	5.97	42.80
PIXE	0.6	Cr	6	5.00	1.2	4.85	14.14
NAA	120	Cr	6	2.22	1	1.98	472.36
NAA	3	Cr	15	0.94	1.1	#NUM!	
NAA	100	Cr	15			0.94	88.36
PIXE	1.85	Cr	6			4.71	41.04
μ PIXE	0.00566	Cr	12			1.91	0.02
μ PIXE	0.00023	Cr	12			3.56	0.00
NAA	10	Cr	6			4.11	168.92
NAA	120	Cs	6	18.20	20.5	#NUM!	
NAA	120	Cu	6	21.26	24.16	#NUM!	
SRXRF	0.0001	Cu	6	12.70	5	11.67	0.01
SRXRF	0.0001	Cu	9	11.30	5	10.13	0.01
PIXE	0.6	Cu	6	10.70	5	9.46	53.69
NAA	120	Eu	6	21.54	22.6	#NUM!	
NAA	1.2	Eu	12	12.80	10	7.99	76.61
SRXRF	0.0001	Fe	6	11.80	0.7	11.78	0.01
μ PIXE	0.00583	Fe	12	9.82	2.11	9.59	0.54
SRXRF	0.0001	Fe	9	9.60	0.7	9.57	0.01
μ PIXE	0.00024	Fe	12	8.64	3.28	7.99	0.02
NAA	1.2	Fe	4	7.10	5.8	4.10	20.12
PIXE	0.6	Fe	6	3.60	0.7	3.53	7.48
PIXE	1.907	Fe	6	3.40	2.1	2.67	13.64
NAA	3	Fe	15	3.25	2.98	1.29	4.99
NAA	120	Fe	6	1.49	1.1	1.00	120.59
NAA	100	Fe	15			2.25	506.25
NAA	10	Fe (1099)	6			1.88	35.34
NAA	10	Fe (1291)	6			5.22	272.48
NAA	120	Hf	6	1.00	26	#NUM!	

PIXE	0.6	Hg	6	13.60	6	12.20	89.38
SRXRF	0.0001	Hg	6	8.60	3	8.06	0.01
SRXRF	0.0001	Hg	9	6.00	3	5.20	0.00
NAA	100	Hg	15	1.54		1.54	237.16
NAA	10	Hg	10	1.34		1.34	18.01
NAA	10	Hg (145)	6			16.64	2768.90
NAA	10	Hg (68)	6			16.27	2647.13
NAA	10	Hg (77)	6			18.14	3290.60
SRXRF	0.0001	K	6	11.20	1.7	11.07	0.01
PIXE	0.6	K	6	10.20	5	8.89	47.42
NAA	1.2	K	6	4.90	2.5	4.21	21.31
SRXRF	0.0001	K	9	3.60	1.7	3.17	0.00
NAA	120	K	6	2.82	1.7	2.25	608.58
NAA	3	K	15	2.43	1.21	2.10	13.26
μ PIXE	0.00019	K	12	1.25	0.64	1.07	0.00
μ PIXE	0.00474	K	12	1.09	0.47	0.98	0.00
NAA	10	K	6	0.68		0.68	4.62
NAA	120	La	6	19.54	5.28	18.94	43057.51
NAA	10	Mg	10	3.45		3.45	119.0465
SRXRF	0.0001	Mn	6	22.20	3.5	21.92	0.048059
SRXRF	0.0001	Mn	9	19.50	3.5	19.18	0.0368
μ PIXE	0.00575	Mn	12	6.70	4.01	5.37	0.165657
μ PIXE	0.00024	Mn	12	5.41		5.41	0.007024
PIXE	0.6	Mn	6	3.10	1.5	2.71	4.416
NAA	10	Mn	10	2.90		2.90	84.21264
NAA	3	Mn	15	2.39	1.086	2.13	13.6268
NAA	120	Mn	6	2.38	1.1	2.11	534.0924
SRXRF	0.0001	Mo	9	19.20	8	17.45	0.030464
SRXRF	0.0001	Mo	6	9.60	8	5.31	0.002816
NAA	120	Mo	6	5.30	13	#NUM!	
NAA	1.2	Na	12	32.50	20	25.62	787.5
NAA	10	Na	10	2.23		2.23	49.90401
NAA	120	Na	6	1.26	1.1	0.62	45.91478
NAA	3	Na	15	1.26	1.07	0.67	1.338624
NAA	100	Na	15			1.14	129.96
NAA	10	Na	6			0.54	2.916
NAA	3	Ni	15	6.62	7.88	#NUM!	
PIXE	0.6	Ni	6	6.50	1.5	6.32	24
SRXRF	0.0001	Ni	9	6.50	0.5	6.48	0.0042

SRXRF	0.0001	Ni	6	5.00	0.5	4.97	0.002475
NAA	120	Ni	6	1.53	2.2	#NUM!	
μ PIXE	0.00011	P	12	1.18	0.57	1.03	0.000117
μ PIXE	0.00256	P	12	1.13	0.43	1.04	0.002796
SRXRF	0.0001	Pb	6	10.30	1.5	10.19	0.010384
PIXE	0.6	Pb	6	9.40	2	9.18	50.616
SRXRF	0.0001	Pb	9	9.20	1.5	9.08	0.008239
SRXRF	0.0001	Rb	9	60.60	10	59.77	0.357236
SRXRF	0.0001	Rb	6	21.60	10	19.15	0.036656
NAA	120	Rb	6	5.02	4.78	1.52	277.8199
PIXE	0.6	S	6	27.60	4	27.31	447.456
μ PIXE	0.00335	S	12	1.32	0.49	1.23	0.005033
μ PIXE	1.096	S	6	1.27	0.81	0.98	1.048653
μ PIXE	0.00014	S	12	1.25	0.67	1.06	0.000156
NAA	120	Sb	6	2.60	2.1	1.53	281.5292
NAA	120	Sc	6	11.37	1.85	11.22	15111.42
PIXE	0.6	Sr	6	43.70	5	43.41	1130.814
NAA	120	Ti	6	35.06	17.6	30.33	110364.4
PIXE	0.6	Ti	6	27.10	5	26.63	425.646
NAA	10	V	10	5.60		5.60	313.2348
NAA	120	V	6	4.14	5.216	#NUM!	
SRXRF	0.0001	Zn	9	12.20	1	12.16	0.01
SRXRF	0.0001	Zn	6	4.70	1	4.59	0.00
PIXE	0.6	Zn	6	4.30	2	3.81	8.69
NAA	1.2	Zn	5	3.04	2.2	2.10	5.28
NAA	120	Zn	6	2.33	1	2.10	528.75
NAA	3	Zn	15	2.30	1.68	1.57	7.36
NAA	100	Zn	15			1.75	306.25

Annex 2

FIGURES

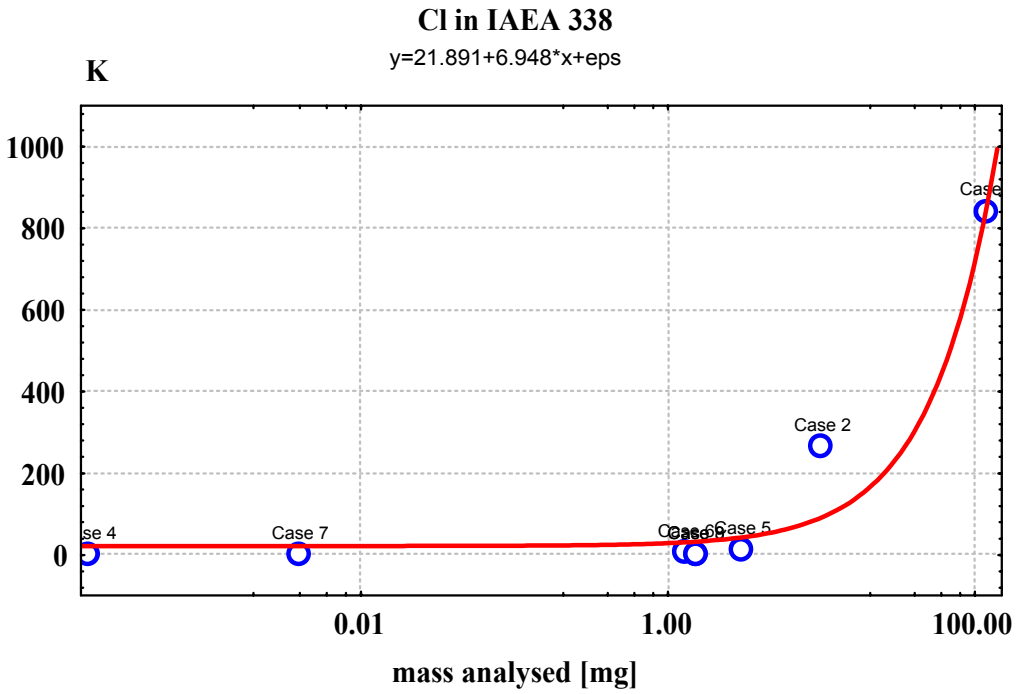

FIG. 1. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Cl by different techniques in IAEA 338.*

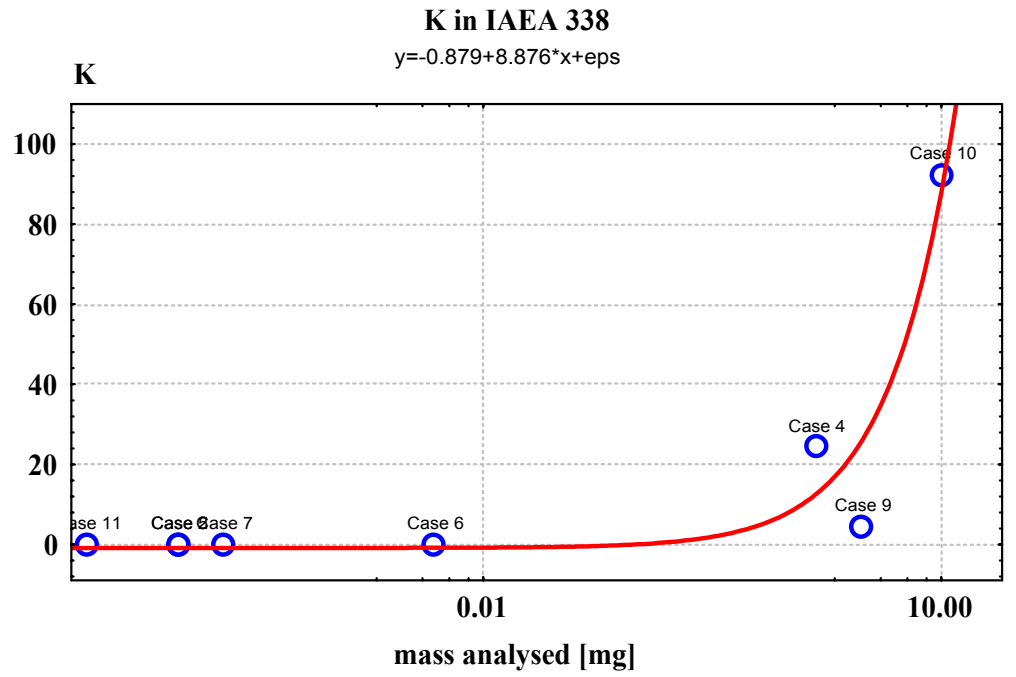

FIG. 2. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of K by different techniques in IAEA 338.*

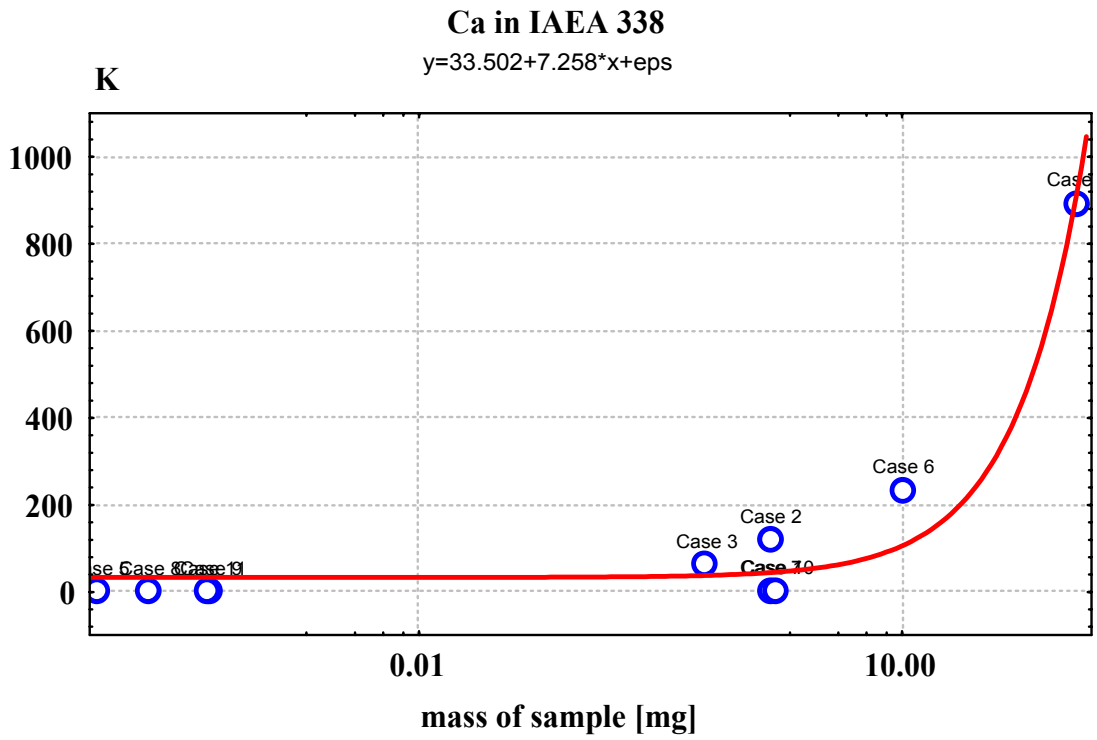

FIG. 3. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Ca by different techniques in IAEA 338.*

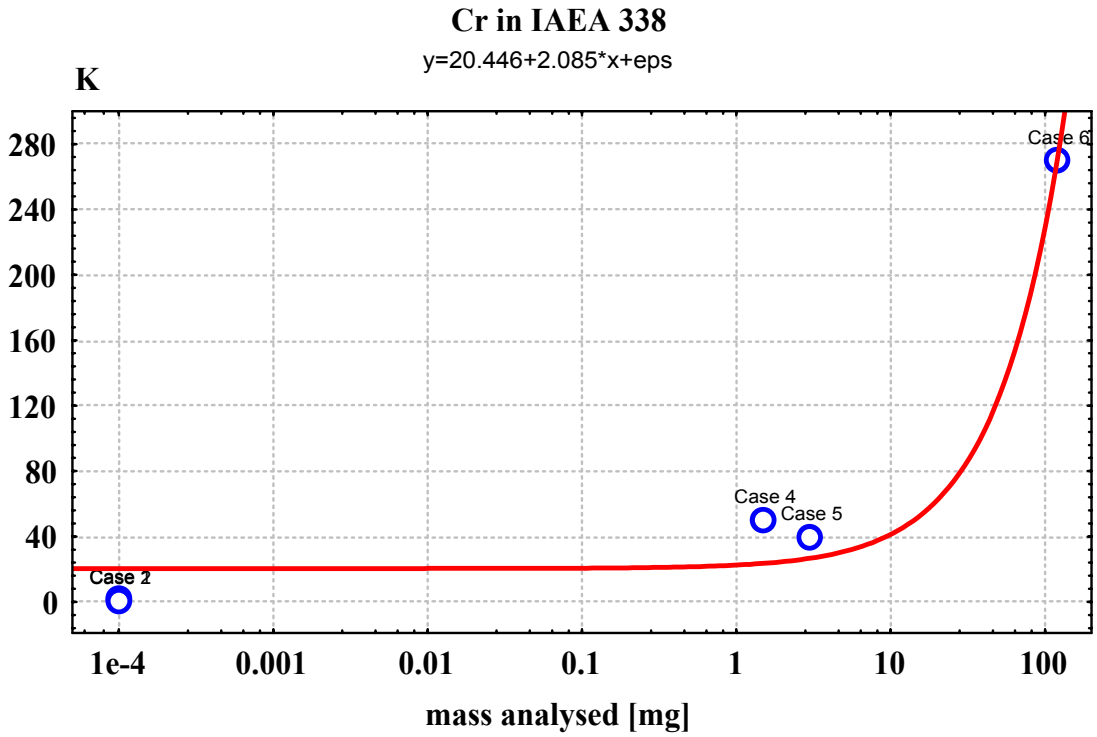

FIG. 4. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Cr by different techniques in IAEA 338.*

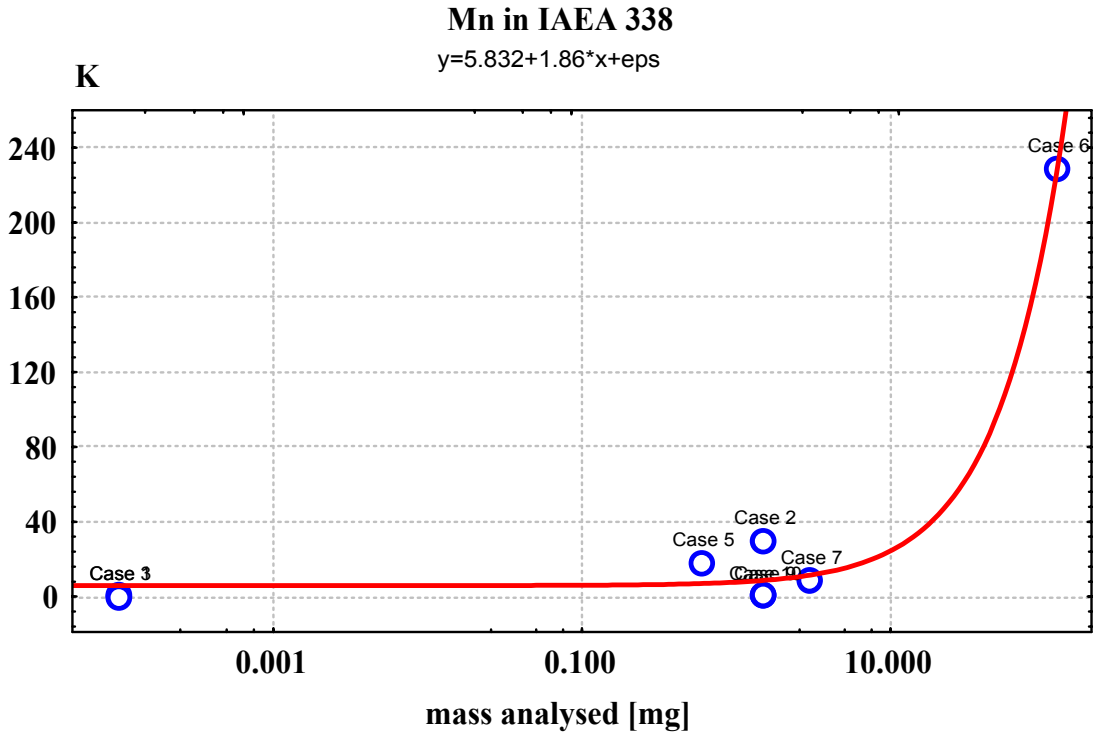

FIG. 5. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Mn by different techniques in IAEA 338.*

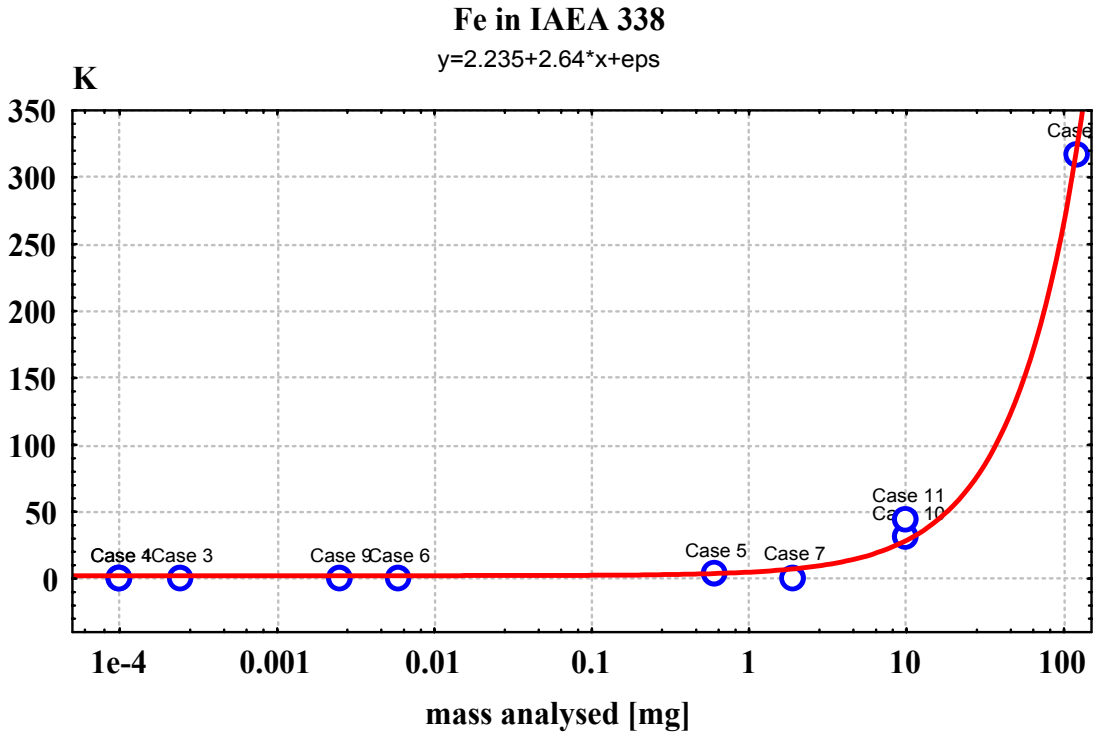

FIG. 6. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Fe by different techniques in IAEA 338.*

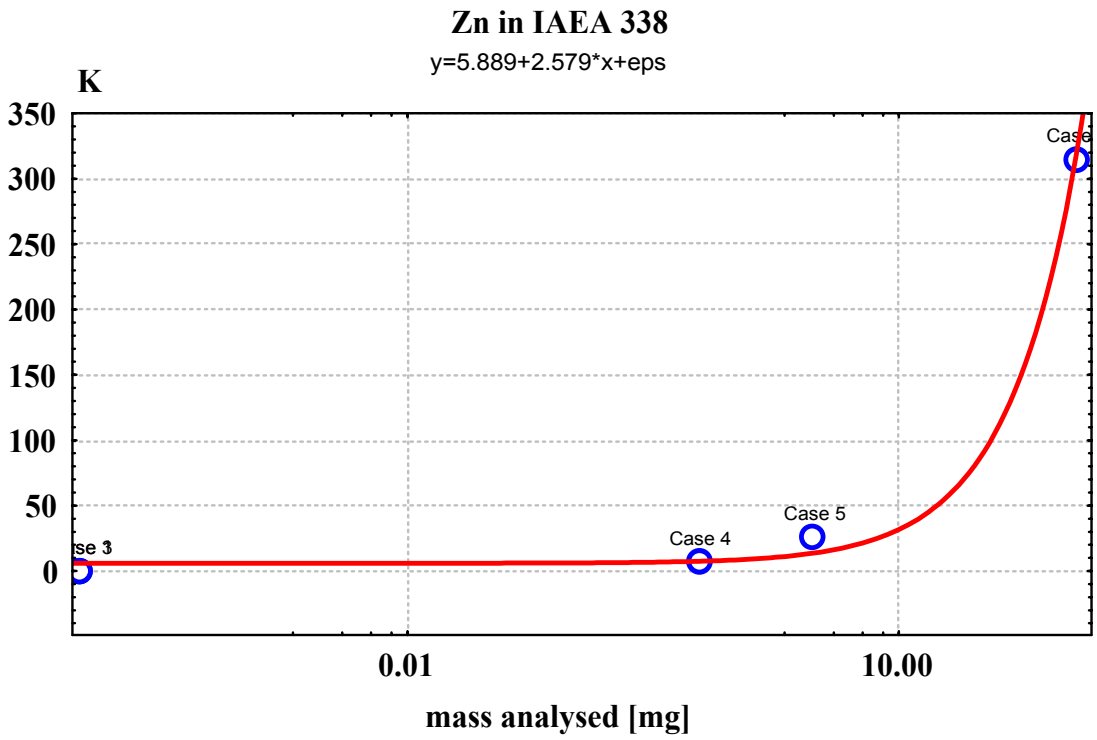

FIG. 7. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Zn by different techniques in IAEA 338.*

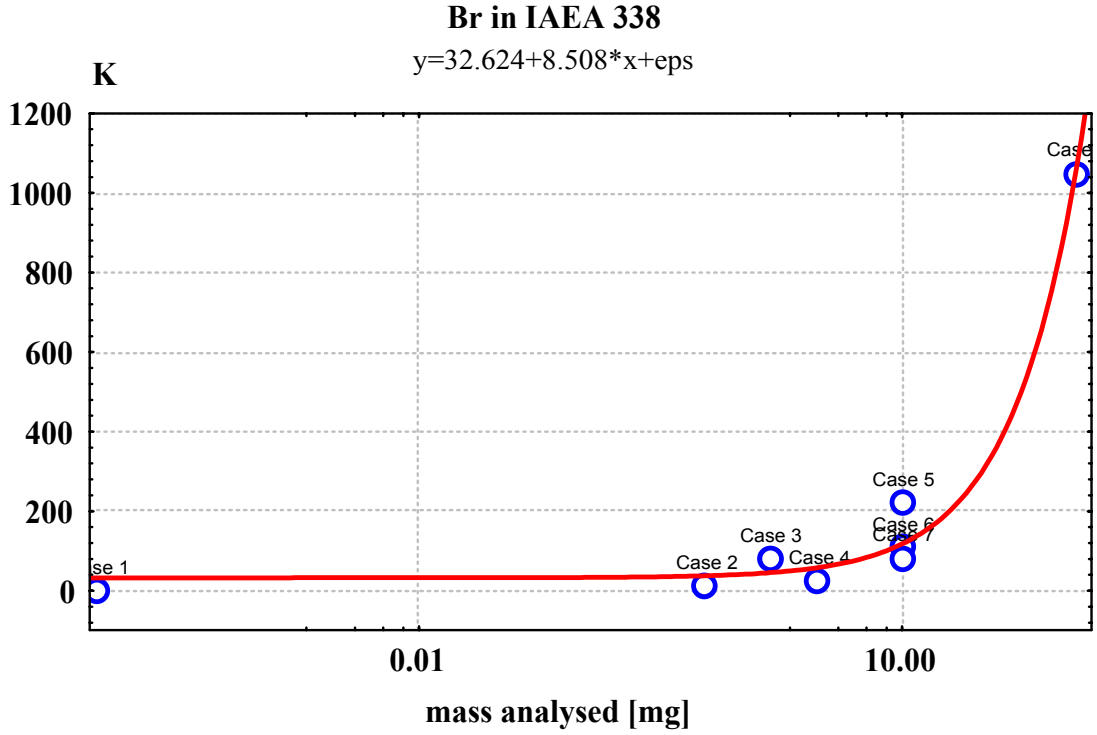

FIG. 8. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Br by different techniques in IAEA 338.*

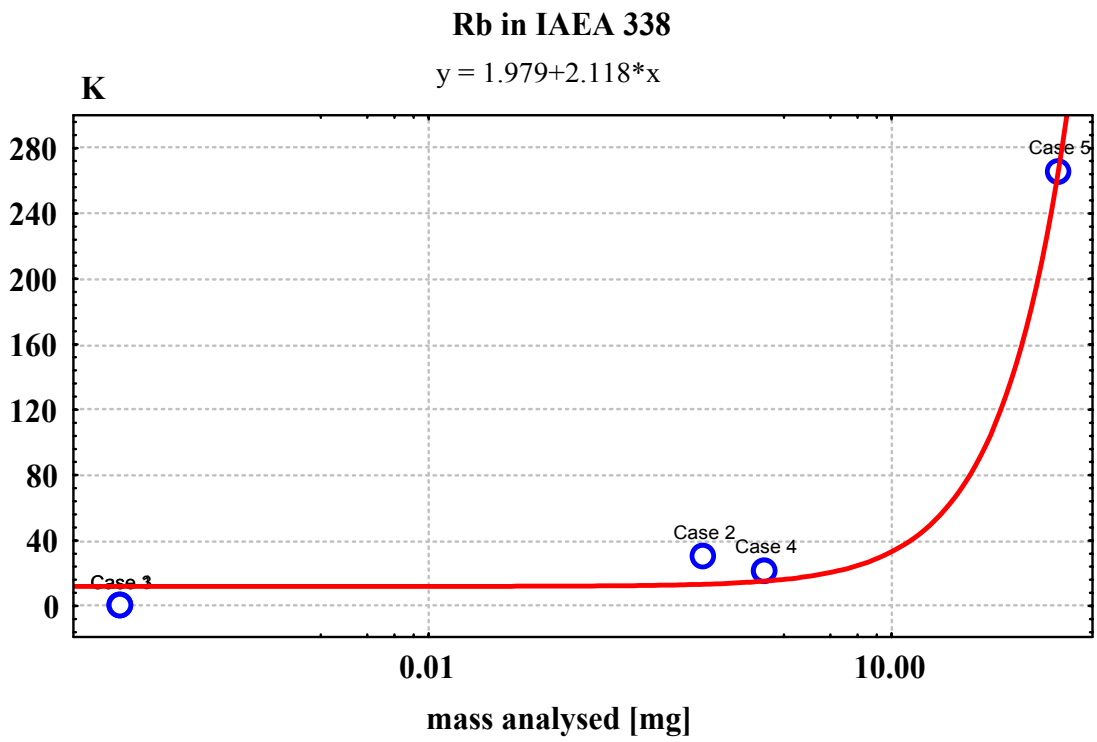

FIG. 9. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Rb by different techniques in IAEA 338.*

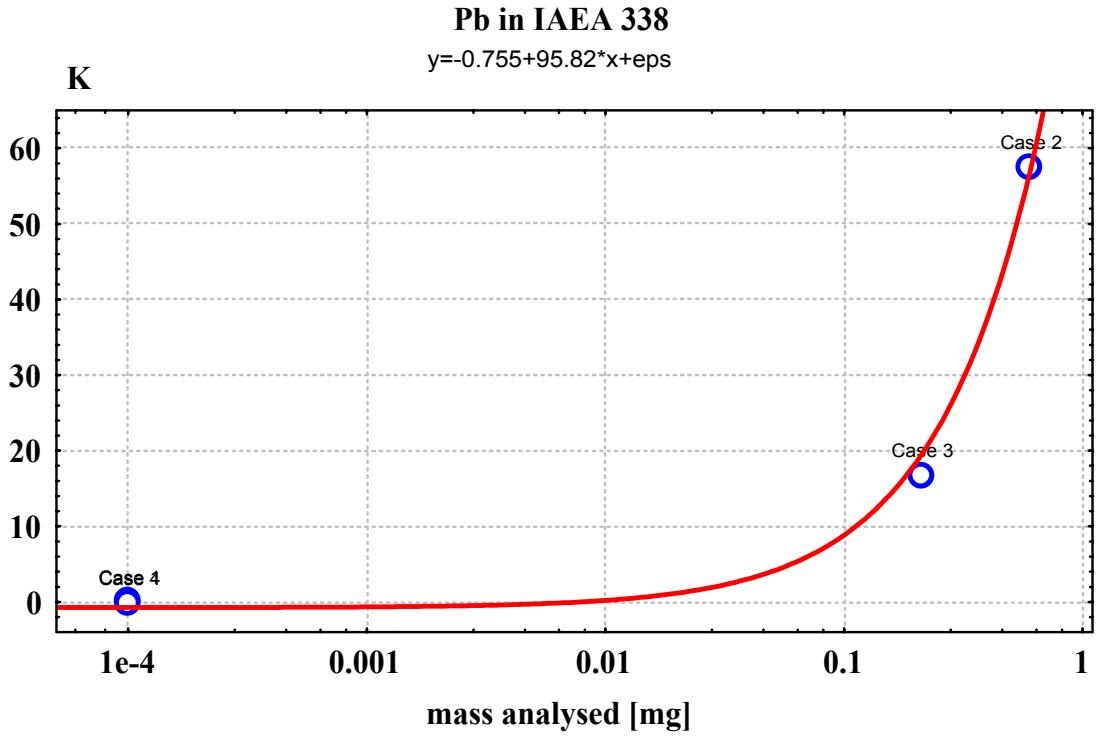

FIG. 10. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Pb by different techniques in IAEA 338.*

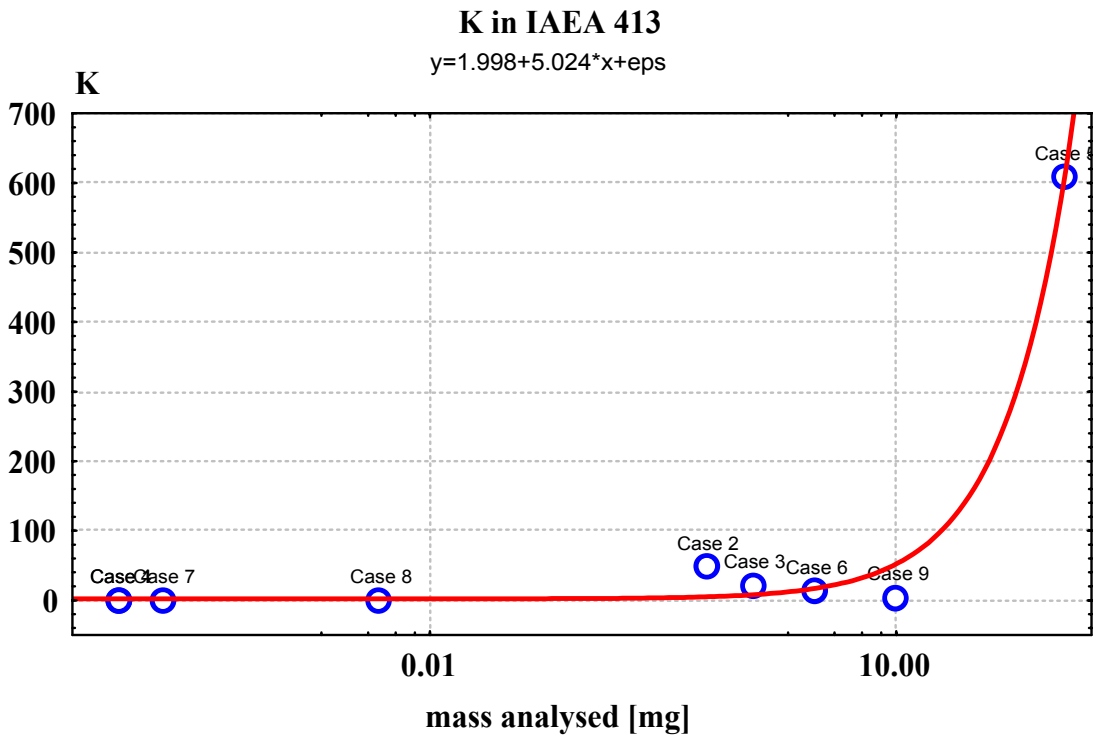

FIG. 11. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of K by different techniques in IAEA 413.*

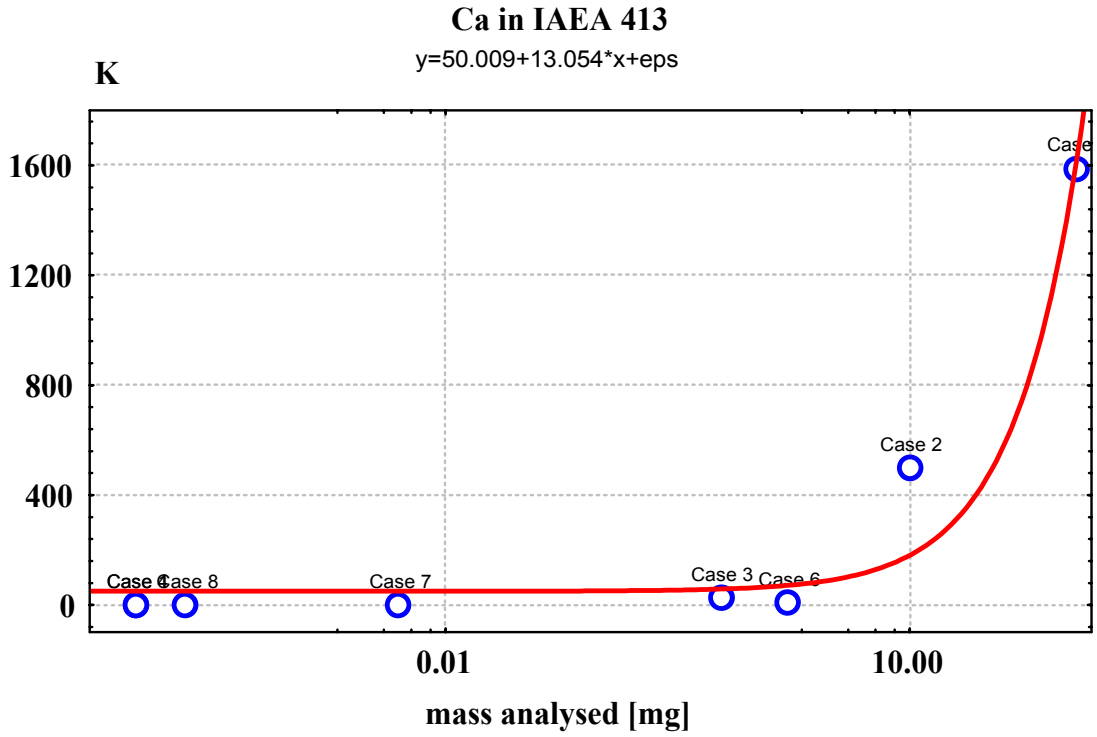

FIG. 12. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Ca by different techniques in IAEA 413.*

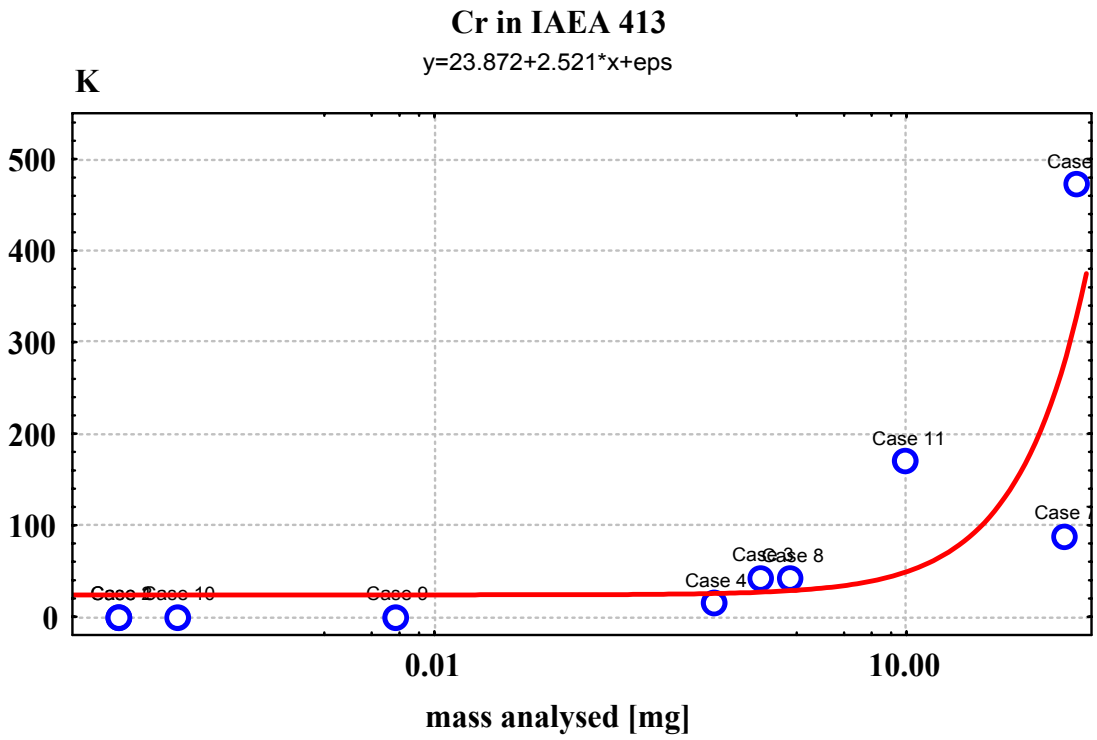

FIG. 13. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Cr by different techniques in IAEA 413.*

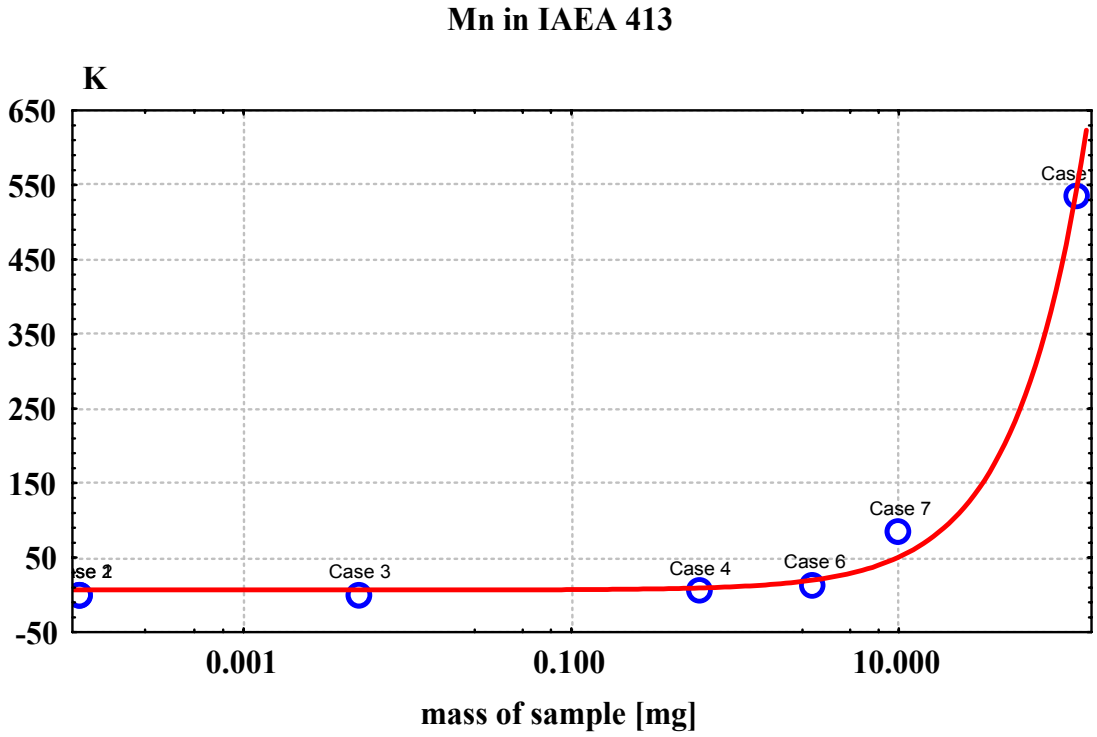

FIG. 14. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Mn by different techniques in IAEA 413.*

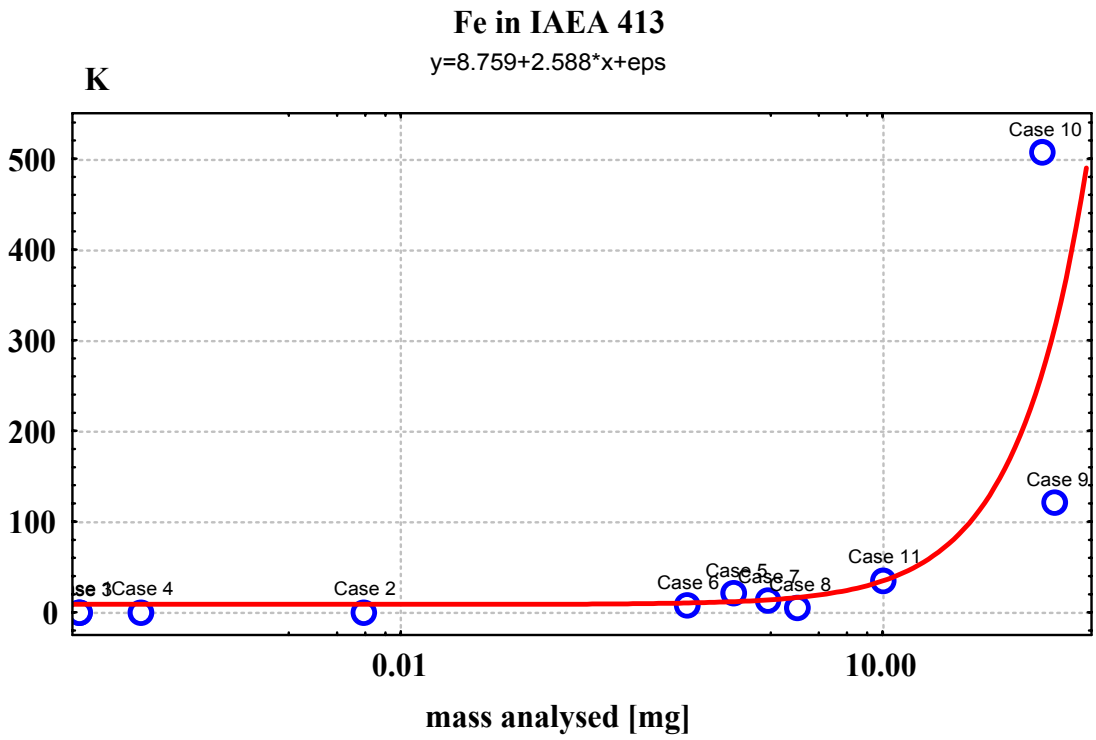

FIG. 15. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Fe by different techniques in IAEA 413.*

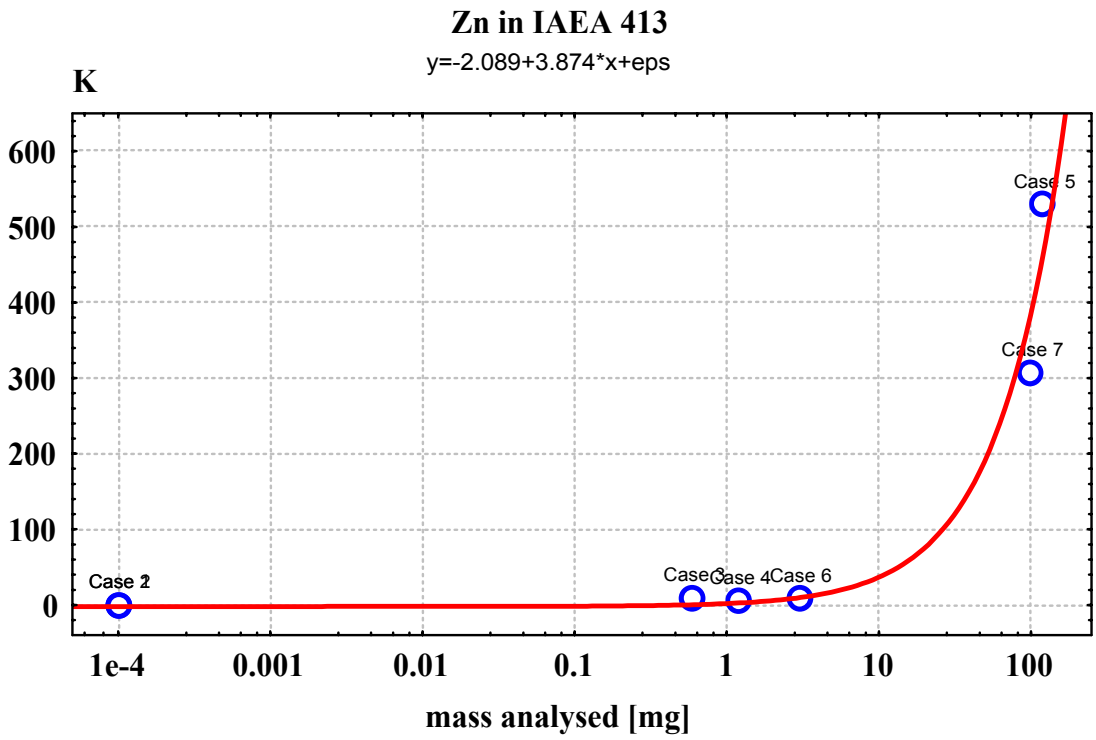

FIG. 16. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Zn by different techniques in IAEA 413.*

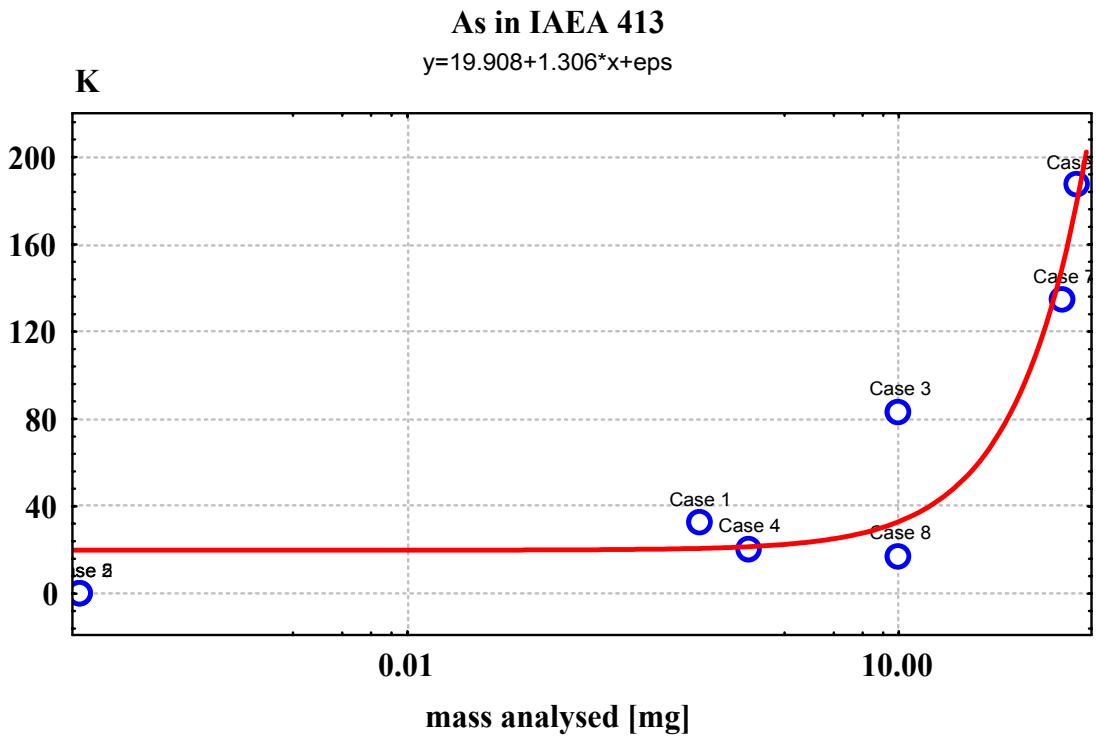

FIG. 17. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of As by different techniques in IAEA 413.*

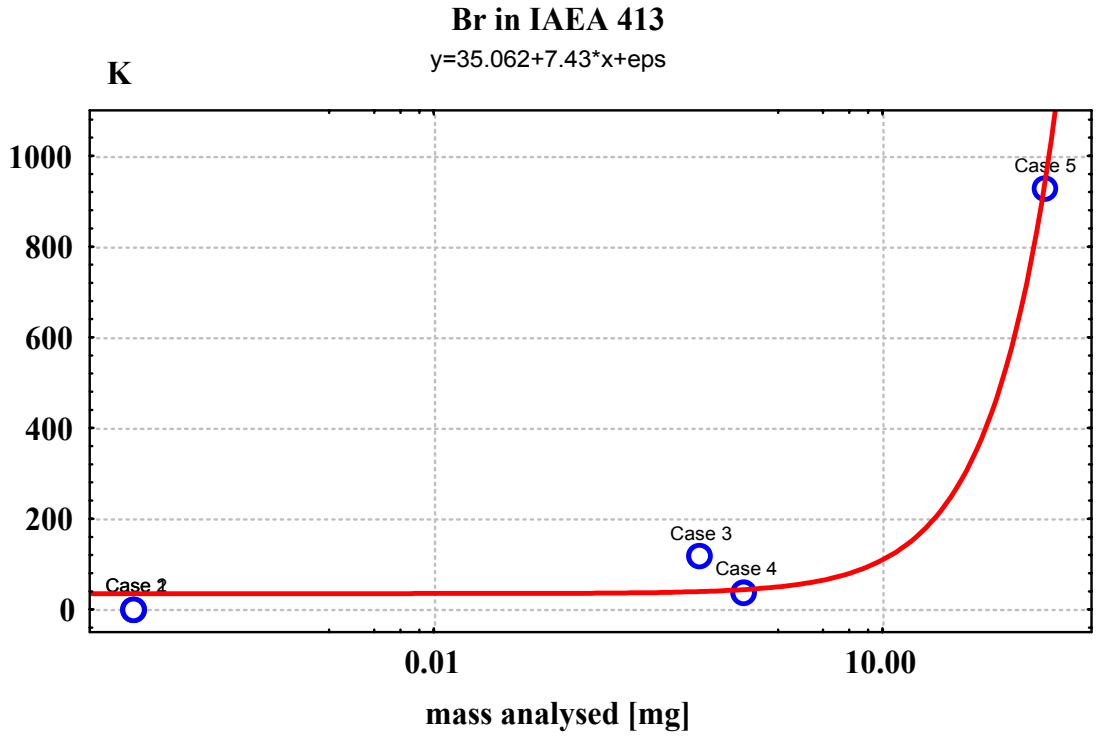

FIG. 18. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Br by different techniques in IAEA 413.*

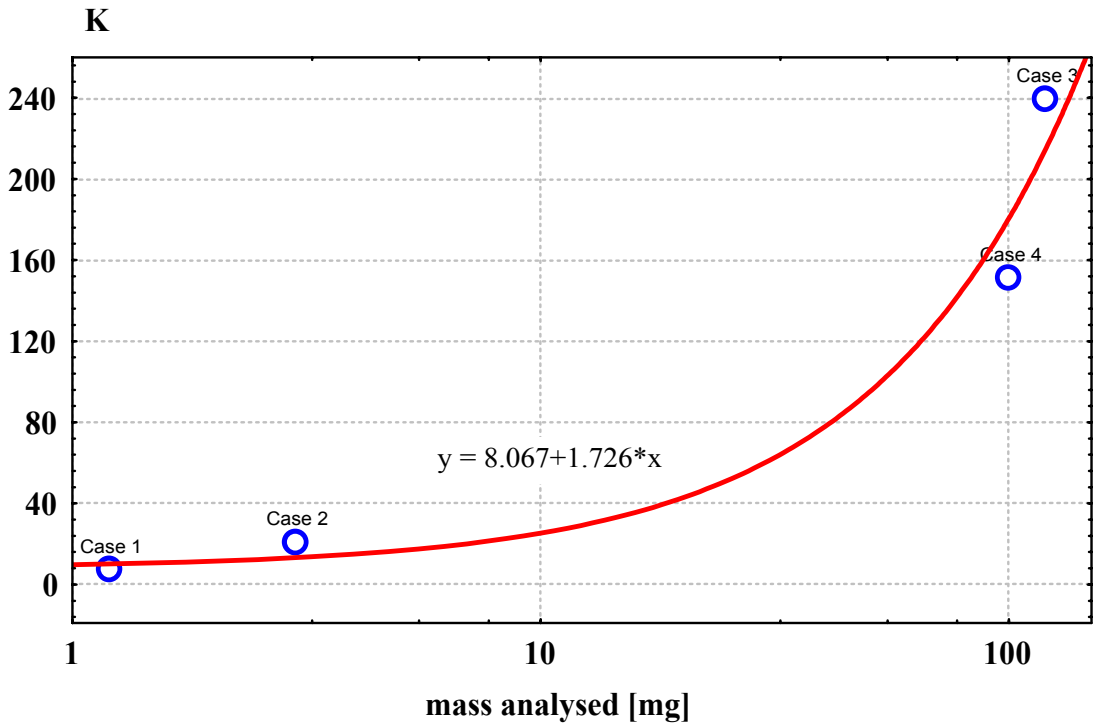

FIG. 19. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Cd by different techniques in IAEA 413.*

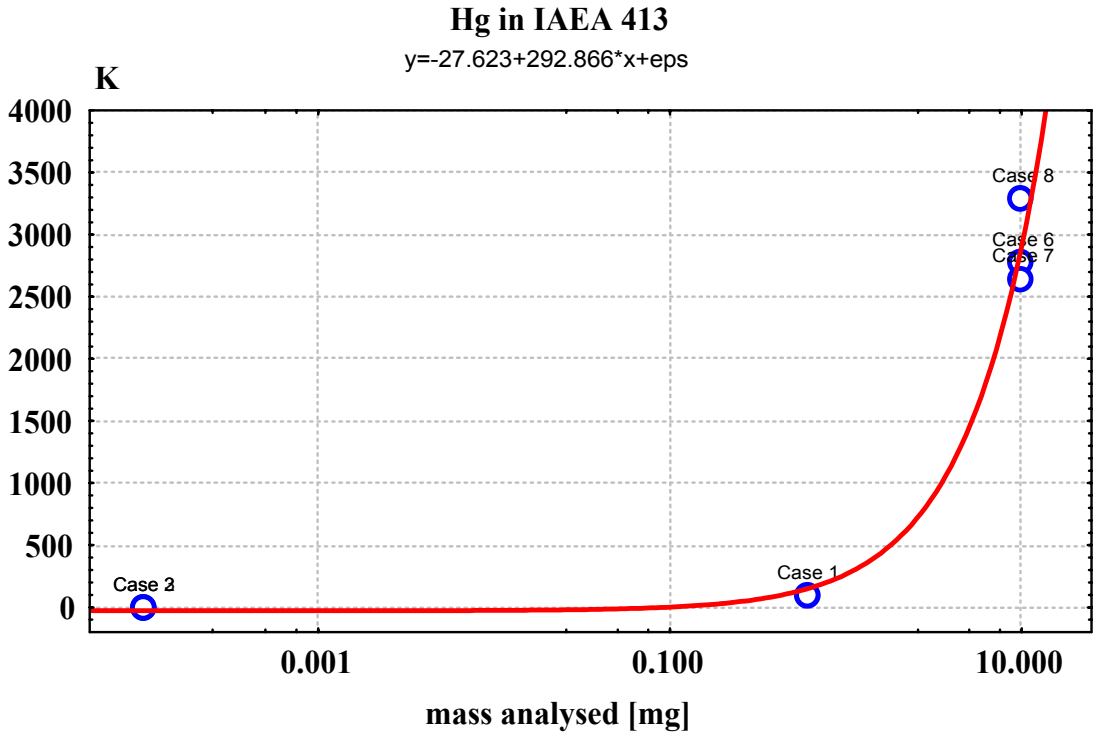

FIG. 20. *Ingamels' sampling constant K_S versus sample mass used for repetitive analysis of Hg by different techniques in IAEA 413.*

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