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ELEMENT CONTENT AND PARTICLE SIZE CHARACTERIZATION OF A MUSSEL CANDIDATE REFERENCE MATERIAL

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ABSTRACT

The use of certified reference materials is an important tool in the quality assurance of analytical measurements. To assure reliability on recently prepared powder reference materials, not only the characterization of the property values of interest and their corresponding uncertainties, but also physical properties such as the particle size distribution must be well evaluated. Narrow particle size distributions are preferable than larger ones; as different size particles may have different analyte content. Due to this fact, the segregation of the coarse and the fine particles in a bottle may lead to inhomogeneity of the reference material, which should be avoided. In this study the element content as well as the particle size distribution of a mussel candidate reference material produced at IPEN - CNEN / SP was investigated. Instrumental Neutron Activation Analysis was applied to the determination of 15 elements in seven fractions of the material with different particle size distributions. Subsamples of the materials were irradiated simultaneously with elemental standards at the IEA-R1 research nuclear reactor and the induced gamma ray energies were measured in a hyperpure germanium detector. Three vials of the candidate reference material and three coarser fractions, collected during the preparation, were analyzed by Laser Diffraction Particle Analysis to determine the particle size distribution. Differences on element content were detected for fractions with different particle size distribution, indicating the importance of particle size control for biological reference materials. From the particle size analysis, Gaussian particle size distribution was observed for the candidate reference material with mean particle size $\mu = 94.6 \pm 0.8$ µm.

1. INTRODUCTION

The use of certified reference materials is an important tool in the quality assurance of analytical measurements as they are used for method validation, equipment calibration and for establishing metrological traceability links of measurement results [1]. To assure reliability on recently prepared powder reference materials, not only the characterization of

the property values of interest and their corresponding uncertainties, but also physical properties such as the particle size distribution must be well evaluated.

Distributions of particles within a narrow range are preferred for reference materials, since different particle sizes in a bottle can lead to segregation of the material, interfering with the homogeneity as different size particles may have different analyte content [2]. The problem is critical for metallic or geological matrix reference materials in which the existence of particles and aggregates with different phases is possible. The problem is less critical in the case of biological reference materials. However, particle size characterization must be performed on new biological reference materials to assure adequate homogeneity.

Besides the use of sieves with various sieve openings, laser diffraction technique was used to characterize the particle size distribution of the mussel candidate reference material.

The characterization of particle size of powders, made by laser diffraction technique, assumes that the pattern of scattering of light, formed on the detector, is the sum of the scattering produced by each sampled particle [3]. The deconvolution of the resulting pattern provides information about the size of the particles. The technique has advantages such as speed of analysis, ease of use and relatively simple sample preparation. As limitations, the performance depends heavily on the optical characteristics of the equipment, the technique does not distinguish between particle clusters and significant bias can be obtained if the shape of the particles has very large deviation from spherical form [4]

In this study the particle size distribution of a mussel candidate reference material produced at IPEN - CNEN / SP was investigated by Laser Diffraction Particle Size Analysis. Instrumental Neutron Activation Analysis was also applied to the determination of 15 elements in seven fractions of the material with different particle size distributions to investigate whether there are element content differences among the different portions of the material.

2. EXPERIMENTAL

2.1. Reference Material Particle Size Adjustment

The preparation of the mussel reference material batch is described in detail elsewhere [5, 6]. After the freeze-drying process, 250 mL of the material was grinded in a domestic blender with titanium blades for 1 min at velocity 1 of the blender. This protocol led to homogenization of the grinding process and avoided the overheating of the blender. Afterwards, the material was sequentially sieved in polyester sieves with different sieve openings (Tenyl Tecidos Técnicos Ltda.): 500 µm (32 mesh), 354 µm (42 mesh), 250 µm (60 mesh), 177 µm (80 mesh), 149 µm (100 mesh), 125 µm (115 mesh) and 105 µm (150 mesh). Part of the material that did not pass one sieve was once again subjected to the grinding and sieving processes up to three times. The fractions with particle size more than 105 µm were not considered for the preparation of the reference material.

Table 1 lists the different particle size fractions analyzed in this study as well as the used certified reference materials. All particle size fractions were analyzed by INAA. Due to instrumental limitations, only the fractions bellow 177 µm were analyzed by laser diffraction.

A stratified random scheme was used for selection of the bottles of the candidate reference material for analysis.

Table 1 List of materials analyzed in this study

Material	Sample	Description	INAA	Particle size
Mussel material fraction	M32	> 500 µm	X	-
	M42	354 – 500 µm	X	-
	M60	250 – 354 µm	X	-
	M80	177 – 250 µm	X	-
	M100	149 – 177 µm	X	X
	M115	125 – 149 µm	X	X
	M150	105 – 149 µm	X	X
Mussel candidate reference material	87	Bottle	X	-
	52	Bottle	-	X
	100	Bottle	-	X
	162	Bottle	-	X
Certified Reference Material	NIST SRM 2976	Mussel tissue	-	X
	NIST SRM 1566b	Oyster tissue	-	X
	DORM-2	Dogfish muscle	-	X
	IAEA 407	Fish tissue	-	X

2.2. Instrumental Neutron Activation Analysis

The comparative method of Instrumental Neutron Activation Analysis (INAA) was used to the determination of Ag, As, Br, Co, Cr, Cs, Eu, Fe, La, Na. Rb, Se, Th and Zn in different particle size portions of the mussel candidate reference material and in one bottle of the candidate reference material.

2.2.1. Sample and elemental standards preparation

Subsamples of approximately 0.150 g were weighed in properly cleaned polyethylene bags using a Shimadzu AEM-5200 analytical balance. Elemental standards were prepared by pipetting Spex standard element solutions onto Whatman paper filters, using variable volume pipettes (Eppendorf or Jencons). For some elements, the original solution was diluted in volumetric flasks prior to pipetting. After drying, paper filters were kept in polyethylene vials with the same geometry as for the samples. Three subsamples were taken from each bottle for analysis.

2.2.2. Irradiation and element determination

Subsamples and elemental standards were irradiated simultaneously for 8 hours at 10^{12} n cm^{-2} s^{-1} thermal neutron flux of the IEA-R1 Nuclear Research Reactor at IPEN-CNEN/SP. ^{82}Br and ^{140}La radionuclides were measured for 1.5 hours, after a 7-day decay period, while ^{110m}Ag, ^{60}Co, ^{134}Cs, ^{152}Eu, ^{59}Fe, ^{233}Pa (for Th determination), ^{75}Se and ^{46}Sc radionuclides were measured for 10 hours, after a 15-day decay period. Gamma ray measurements were performed using a GC2018 Canberra HPGe detector coupled to a Canberra DSA-1000 multichannel analyzer. Gamma ray spectra were collected and processed using a Canberra Genie 2000 version 3.1 spectroscopy software. Element content calculations were carried out using a Microsoft Excel spreadsheet for suitable radionuclide photopeak energies.

2.3. Particle Size Distribution

Subsamples of bottles number 52, 100 and 162 of the mussel candidate reference material, subsamples with coarser granulometries and four different marine origin certified reference materials (NIST SRM 2976, NIST SRM 1566b, DORM-2 and IAEA 407) were characterized by laser diffraction technique using the particle size analyzer CILAS model 1064L, at IPEN – CNEN/SP. Subsamples were dispersed in isopropanol (P. A., Casa Americana) until approximately 12 % obscuration was obtained at the equipment for the determination of the particle size distribution curves.

3. RESULTS AND DISCUSSION

3.1. Instrumental Neutron Activation Analysis

Table 2 presents the element mass fractions obtained by INAA for Ag, As, Br, Co, Cr, Cs, Eu, Fe, La, Na. Rb, Se, Th and Zn in different particle size portions of the mussel candidate reference material and in one bottle of the candidate reference material. The different particle size fractions results were normalized to the results obtained for bottle 87 and are presented in graphical form for selected elements in Figure 1. For the majority of the elements, it was observed lower element content for the coarser fractions if compared to the results obtained for the candidate reference material. In addition, a trend of decrease in mass fraction with increasing sample particle size was observed for all elements. With the exceptions of As, Br and Na, this trend was more pronounced for the coarser fraction, M32. These observations demonstrate the need for tight control of the particle size distribution of candidate reference materials in order to obtain reference materials without homogeneity problems during the sample intake by end-users.

Table 2 Mass fraction, mg kg^{-1}, obtained by INAA for different fractions of the mussel candidate reference material*

	Mussel material fraction							Bottle 87
	M32	M42	M60	M80	M100	M115	M150	
Ag	0.83±0,09	1.64±0.09	1.73±0.15	2.15±0.04	1.86±0.07	2.15±0.28	2.35±0.21	2.46±0.07
As	10.64±0.17	11.75±0.18	8.77±2.0	10.46±1.7	ND	ND	ND	13.76±0.42
Br	182±14	179±3	230±39	223±25	223±30	ND	ND	200±13
Co	0.583±0.036	0.692±0.010	0.701±0.033	0.767±0.020	0.820±0.086	0.806±0.086	0.849±0.084	0.837±0.034
Cr	0.525±0.068	0.891±0.017	0.738±0.055	1.008±0.054	0.848±0.11	1.000±0.091	0.981±0.048	1.100±0.086
Cs	0.041±0.001	0.075±0.007	0.067±0.017	0.095±0.003	0.091±0.014	0.091±0.013	0.103±0.006	0.102±0.015
Eu	0.0263±0.0020	0.0429±0.0023	0.0420±0.0028	0.0447±0.0039	0.0427±0.0008	0.0464±0.0074	0.0471±0.0049	0.0493±0.0008
Fe	234±20	411±5	374±13	502±18	403±15	466±80	528±29	604±28
La	0.284±0.03	0.459±0.03	0.567±0.17	0.592±0.07	0.481±0.13	ND	ND	0.645±0.043
Na%	1.700±0.011	1.693±0.050	1.858±0.088	1.692±0.011	1.894±0.003	1.903±0.034	1.905±0.043	1.886±0.051
Rb	3.13±0.50	3.62±0.29	3.74±0.19	4.18±0.24	3.96±0.15	3.99±0.24	4.51±0.49	5.015±0.263
Sc	0.0712±0.005	0.1316±0.0040	0.1206±0.0059	0.1601±0.0063	0.1302±0.0043	0.1522±0.0013	0.1740±0.023	0.198±0.014
Se	3.15±0.07	3.47±0.11	3.88±0.14	4.00±0.10	4.04±0.29	4.14±0.10	4.28±0.44	4.339±0.040
Th	0.084±0.004	0.168±0.002	0.164±0.017	0.203±0.005	0.176±0.009	0.219±0.031	0.227±0.032	0.270±0.023
Zn	80.2±6.3	91.7±0.8	95.8±1.0	102.2±2.8	99.4±5.6	103.0±6.8	108.6±0.9	114.5±4.8

*Mean and standard deviation, $n = 3$; ND – not determined.

3.2. Particle size distribution

In Table 3, the results of a screening performed on the bulk mussel material before bottling are presented. It was observed that 15 % of the material has a particle size distribution less than 74 µm indicating that the size distribution of the material is narrow, between 74 µm and 105 µm. The corresponding laser diffraction results, presented afterwards, are consistent with this observation.

Table 3 Mussel candidate reference material sieved fractions

Sieve opening	Sieved sample, g	Mass fraction, %
105 µm (150 mesh)	15.06	100
74 µm (200 mesh)	2.27	15.1
63 µm (250 mesh)	1.52	10.1
53 µm (270 mesh)	1.12	7.4

During the tests performed to characterize the particle size distribution of the mussel reference material by laser diffraction, it was concluded that water would not be used as dispersing agent, since cluster formation was observed with that agent. Anhydrous isopropanol proved to be a suitable dispersing agent. In addition, it was observed that sonication and use of additional dispersing agents did not change the profile of the distribution curves and, therefore, were not used in this study.

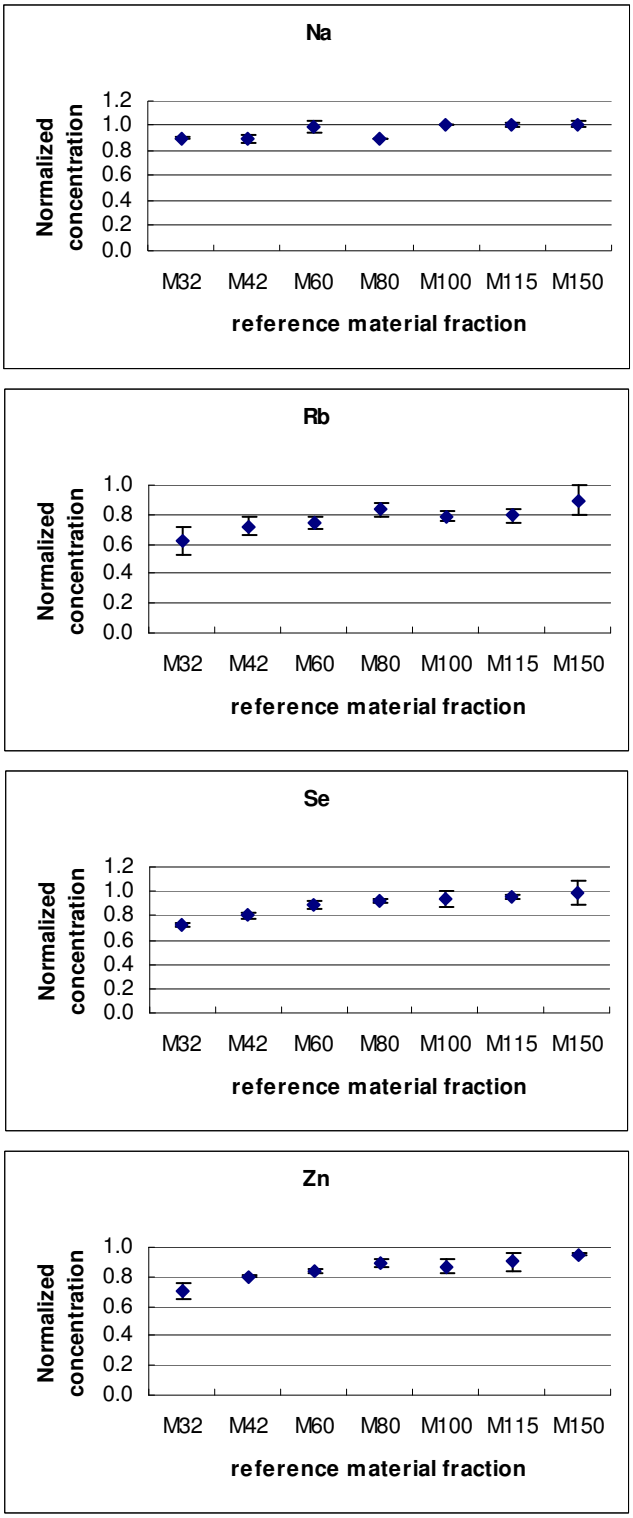

Figure 1. Normalized mass fractions for Na, Rb, Se and Zn obtained by INAA for different fractions of the mussel candidate reference material.

Figure 2 shows the typical particle size distribution profile of the mussel candidate reference material obtained in volume/undersize. Figure 3 shows the comparison of distributions of different portions of the material. It was observed that the particle size distribution of the various fractions coincided with the expected ones, considering the sieve openings used to obtain the fractions. The mussel candidate reference material has a mean particle size of $\mu = 94.6 \pm 0.8$ µm, with a Gaussian distribution ($R^2 = 0.97$ for Gaussian curve fitting), as shown in Figure 4. This result is very important because size distributions with very wide deviations from normal or multimodal distributions can impair the homogeneity of candidate reference materials [2].

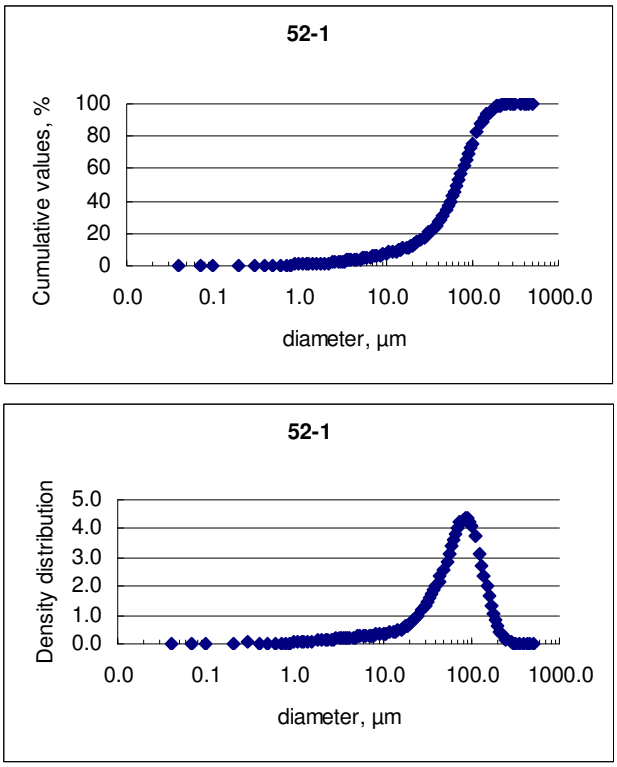

Figure 2. Particle size distribution for bottle 52 of the mussel reference material.

Figure 5 shows the overlapping particle size distribution of three bottles of mussel candidate reference material, chosen from the beginning, middle and end of the bottling process. Great similarity was observed among the particle size distributions of the bottles. This fact proves that the bottling process was satisfactory, showing that there is no major differences between bottles of the material with respect to particle size.

The comparison with the results obtained for the certified reference materials with similar matrix showed that the mussel candidate reference material presents particle size distribution similar to the fish homogenate (IAEA-407), as shown in Figure 6. It was also noted that the

other certified reference materials present finer grain size distributions, the result of applying different milling process in the production of these materials.

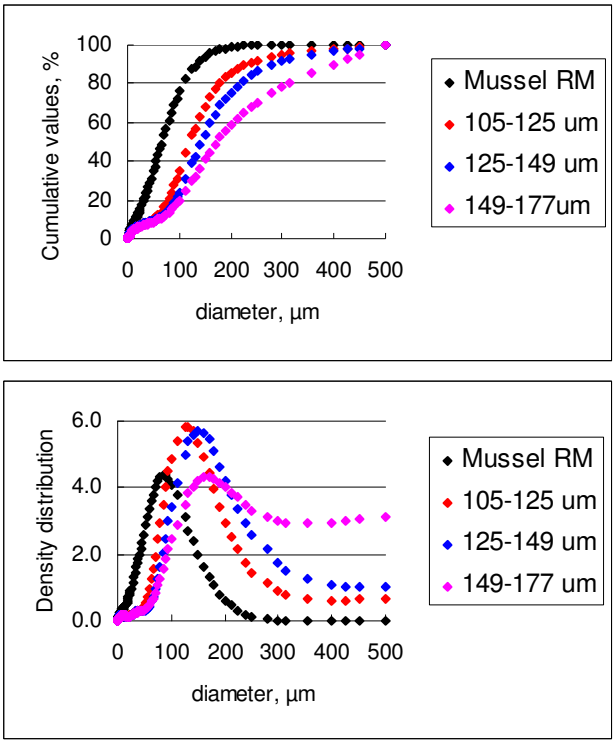

Figure 3. Particle size distribution for different fractions of the mussel reference material.

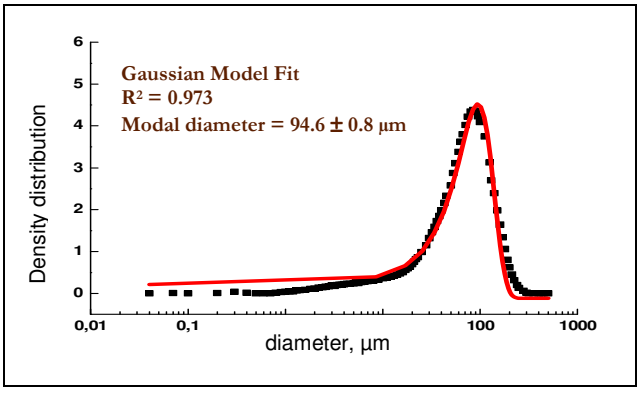

Figure 4. Gaussian model fitting to the particle size distribution of bottle 52 of the mussel reference material.

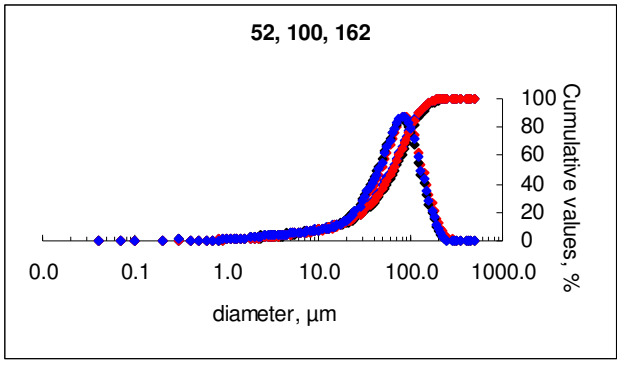

Figure 5. Particle size distribution for bottles 52, 100 and 162 of the mussel reference material.

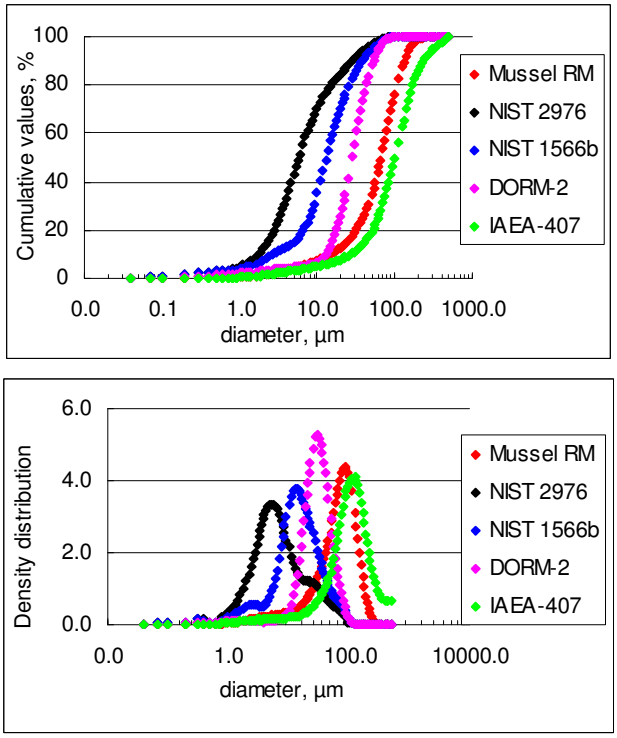

Figure 6. Particle size distribution comparison for the mussel reference material and different certified reference materials.

Considering the particle size distribution characterization presented in this paper, it was concluded that batch of the mussel candidate reference material has suitable particle size distribution to be used as certified reference material.

4. CONCLUSIONS

In this study a Gaussian particle size distribution was observed for a mussel candidate reference material, with mean particle size $\mu = 94.6 \pm 0.8$ µm. Such distribution is considered suitable for the material to be used as a certified reference material. Differences on element content were detected for fractions with different particle size distribution, indicating the importance of particle size control in the production of biological reference materials.

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