J Radioanal Nucl Chem (2015) 303:783–788
DOI 10.1007/s10967-014-3379-4

Element content and particle size characterization of a mussel candidate reference material

**E. G. Moreira · M. B. A. Vasconcellos ·
R. G. Santos · J. R. Martinelli**

Received: 21 May 2014 /

Abstract The element content as well as the particle size distribution of a new mussel candidate reference material was investigated. Instrumental Neutron Activation Analysis was applied for the determination of 15 elements in seven fractions of the material. Three vials of the candidate reference material and three coarser fractions 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 distributions, indicating the importance of particle size control for biological reference materials.

Keywords Reference material · Trace elements · Particle size distribution · INAA · Laser diffraction · Mussel

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 can lead to segregation of the material in the bottle of a reference material. If it occurs, it may interfere with the homogeneity of the reference material as different size particles may have different contents of the measurands of interest [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. Even though the problem is less critical in the case of biological matrix reference materials, particle size characterization must be performed on new biological reference materials to assure adequate homogeneity.

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]. Studies on particle size distributions are very important in technological areas such physical characterization and biological properties of nanomaterials [5–7] and in nuclear materials forensics [8]. The technique is also essential in the investigation of the adverse effects of airborne particulate matter to human health [9, 10], in the characterization of new reference materials [11, 12] and in the production of reference materials for the validation of analytical techniques that require very small sample amount [13–15].

E. G. Moreira (✉) · M. B. A. Vasconcellos · R. G. Santos ·
J. R. Martinelli
Instituto de Pesquisas Energéticas e Nucleares, IPEN-CNEN/SP,
Av. Prof. Lineu Prestes, 2242, São Paulo, SP 05508-000, Brazil
e-mail: emoreira@ipen.br

Table 1 List of materials analyzed in this study

Material	Sample	Description	INAA	Particle size
Mussel material fraction	M32	>500 μm	✔	–
	M42	354–500 μm	✔	–
	M60	250–354 μm	✔	–
	M80	177–250 μm	✔	–
	M100	149–177 μm	✔	✔
	M115	125–149 μm	✔	✔
	M150	105–125 μm	✔	✔
Mussel candidate reference material	87	Bottle	✔	–
	52	Bottle	–	✔
	100	Bottle	–	✔
	162	Bottle	–	✔
Certified reference material	NIST SRM 2976	Mussel tissue	✔	✔
	NIST SRM 1566b	Oyster tissue	✔	✔
	NRCC DORM-2	Dogfish muscle	✔	✔
	NRCC DOLT-3	Dogfish liver	✔	–
	IAEA 407	Fish tissue	–	✔

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 for 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.

Experimental

Reference material particle size adjustment

The preparation of the mussel reference material batch is described in detail elsewhere [16, 17]. 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 overheating of the blender. Afterwards, the material was sequentially sieved in polyester sieves with different sieve openings (Tenyl Technical Fabrics Ltd.): 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 that did not pass the 105 μm sieve 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.

Instrumental neutron activation analysis

The comparative method of instrumental neutron activation analysis (INAA) was used for the determination of Ag, As, Br, Co, Cr, Cs, Eu, Fe, La, Na, Rb, Sc, Se, Th and Zn in different particle size portions of the mussel candidate reference material and in one bottle of the candidate reference material. Suitable certified reference materials of marine origin (NIST SRM 2976, NIST SRM 1566b, NRCC DORM-2 and NRCC DOLT-3) were simultaneously analyzed to check the validation of the applied INAA method.

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). 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.

Subsamples and elemental standards were irradiated simultaneously for 8 h at 10^{12} cm^{-2} s^{-1} thermal neutron flux of the IEA-R1 Nuclear Research Reactor at IPEN—CNEN/SP. ^{76}As, ^{82}Br, ^{140}La and ^{24}Na radionuclides were measured for 1.5 h, after a 7-day decay period, while ^{110m}Ag, ^{60}Co, ^{51}Cr, ^{134}Cs, ^{152}Eu, ^{59}Fe, ^{233}Pa (for Th determination), ^{86}Rb, ^{46}Sc, ^{75}Se and ^{65}Zn radionuclides were measured for 10 h, 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. Measurement uncertainties were also estimated using a Microsoft Excel spreadsheet [18].

Table 2 Mass fraction in mg kg^{-1} (mean values and expanded uncertainties, $k = 2$) obtained by INAA for selected reference materials in comparison to certified values

	Certified reference material							
	NIST SRM 2976		NIST SRM 1566b		NRCC DORM-2		NRCC DOLT-3	
	This study (certified value)	*z*-score	This study (certified value)	*z*-score	This study (certified value)	*z*-score	This study (certified value)	*z*-score
Ag	ND [0.011 ± 0.005]	–	0.664 ± 0.027 (0.666 ± 0.009)	−0.013	ND (0.041 ± 0.013)	–	1.265 ± 0.052 (1.2 ± 0.07)	0.35
As	14.61 ± 0.50 (13.3 ± 1.8)	0.91	6.83 ± 0.23 (7.65 ± 0.65)	−0.91	17.10 ± 0.58 (18.0 ± 1.1)	−0.48	9.86 ± 0.34 (10.2 ± 0.5)	−0.29
Br	221 ± 11 [329 ± 15]	−3.6	44.6 ± 2.2 NC	–	30.5 ± 1.5 NC	–	17.51 ± 0.88 NC	–
Co	0.703 ± 0.018 (0.61 ± 0.02)	0.89	0.3472 ± 0.0090 (0.371 ± 0.009)	−0.35	0.2354 ± 0.0061 (0.182 ± 0.031)	1.4	0.3304 ± 0.0086 NC	–
Cr	0.443 ± 0.067 (0.50 ± 0.16)	−0.64	0.323 ± 0.048 NC	–	30.9 ± 4.6 (34.7 ± 5.5)	−1.2	7.4 ± 1.1 NC	–
Cs	0.0185 ± 0.0017 [0.027 ± 0.001]	−1.4	0.0208 ± 0.0019 NC	–	0.270 ± 0.024 NC	–	0.117 ± 0.011 NC	–
Eu	ND (0.0024 ± 0.0003)	–	0.0157 ± 0.0033 NC	–	0.0117 ± 0.0025 NC	–	0.0467 ± 0.0098 NC	–
Fe	170.2 ± 6.6 (171.0 ± 4.9)	−0.062	209.2 ± 8.2 (205.8 ± 6.8)	0.23	135.9 ± 5.3 (142 ± 10)	−0.57	1496 ± 58 (1484 ± 57)	0.15
Na, %	3.299 ± 0.040 [3.5 ± 0.1]	−1.7	0.3332 ± 0.0040 (0.3297 ± 0.0053)	0.23	0.4946 ± 0.0059 NC	–	0.950 ± 0.011 NC	–
Rb	4.44 ± 0.44 (4.14 ± 0.09)	0.56	2.99 ± 0.30 (3.26 ± 0.14)	−0.63	5.50 ± 0.55 NC	–	3.62 ± 0.36 NC	–
Sc	0.01679 ± 0.00034 (0.0146 ± 0.0003)	0.68	0.0641 ± 0.0013 NC	–	0.002449 ± 0.000049 NC	–	0.00585 ± 0.00012 NC	–
Se	1.91 ± 0.10 (1.80 ± 0.15)	0.40	1.87 ± 0.10 (2.06 ± 0.15)	−0.63	1.482 ± 0.080 (1.40 ± 0.09)	0.38	7.26 ± 0.39 (7.06 ± 0.48)	0.24
Th	0.0177 ± 0.0011 (0.011 ± 0.002)	2.8	0.0298 ± 0.0018 (0.0367 ± 0.0043)	−0.85	0.0172 ± 0.0010 NC	–	ND NC	–
Zn	148.8 ± 2.8 (137 ± 13)	1.1	1285 ± 24 (1424 ± 46)	−1.8	22.53 ± 0.43 (25.6 ± 2.3)	−1.2	94.9 ± 1.8 (86.6 ± 2.4)	1.2

ND not determined, *NC* not certified, [ ] reference values in the certificates

Table 3 Mass fraction in mg kg^{-1} (mean values and expanded uncertainties, $k = 2$) obtained by INAA for different fractions and one bottle of the mussel candidate reference material

	Mussel material fraction							Bottle 87
	M32	M42	M60	M80	M100	M115	M150	
Ag	0.826 ± 0.034	1.637 ± 0.067	1.731 ± 0.071	2.150 ± 0.088	1.860 ± 0.076	2.151 ± 0.088	2.347 ± 0.096	2.46 ± 0.10
As	10.64 ± 0.36	11.75 ± 0.40	8.77 ± 0.30	10.46 ± 0.36	ND	ND	ND	13.76 ± 0.47
Br	182.0 ± 9.1	178.6 ± 9.0	230 ± 12	223 ± 11	223 ± 11	ND	ND	200 ± 10
Co	0.583 ± 0.015	0.692 ± 0.018	0.701 ± 0.018	0.767 ± 0.020	0.820 ± 0.021	0.806 ± 0.021	0.849 ± 0.022	0.837 ± 0.022
Cr	0.525 ± 0.079	0.89 ± 0.13	0.74 ± 0.11	1.01 ± 0.15	0.85 ± 0.13	1.00 ± 0.15	0.98 ± 0.15	1.10 ± 0.17
Cs	0.0409 ± 0.0037	0.0752 ± 0.0068	0.0674 ± 0.0060	0.0946 ± 0.0086	0.0912 ± 0.0082	0.0909 ± 0.0082	0.1031 ± 0.0093	0.1018 ± 0.0092
Eu	0.0263 ± 0.0055	0.0429 ± 0.0090	0.0420 ± 0.0088	0.0447 ± 0.0094	0.0427 ± 0.0090	0.0464 ± 0.010	0.0471 ± 0.0099	0.0493 ± 0.010
Fe	233.6 ± 9.1	411 ± 16	374 ± 15	502 ± 20	403 ± 16	466 ± 18	528 ± 21	604 ± 24
La	0.284 ± 0.021	0.459 ± 0.034	0.567 ± 0.042	0.592 ± 0.044	0.481 ± 0.036	ND	ND	0.645 ± 0.048
Na, %	1.700 ± 0.020	1.693 ± 0.020	1.858 ± 0.022	1.692 ± 0.020	1.894 ± 0.023	1.903 ± 0.023	1.905 ± 0.023	1.886 ± 0.023
Rb	3.13 ± 0.31	3.62 ± 0.36	3.74 ± 0.37	4.18 ± 0.42	3.96 ± 0.40	3.99 ± 0.40	4.51 ± 0.45	5.02 ± 0.50
Sc	0.0712 ± 0.0014	0.1316 ± 0.0026	0.1206 ± 0.0024	0.1601 ± 0.0032	0.1302 ± 0.0026	0.1522 ± 0.0030	0.1740 ± 0.0035	0.1980 ± 0.0040
Se	3.15 ± 0.17	3.47 ± 0.19	3.88 ± 0.21	4.00 ± 0.22	4.04 ± 0.22	4.14 ± 0.22	4.28 ± 0.23	4.34 ± 0.23
Th	0.0844 ± 0.0050	0.168 ± 0.010	0.164 ± 0.010	0.203 ± 0.0012	0.176 ± 0.011	0.219 ± 0.013	0.227 ± 0.014	0.270 ± 0.016
Zn	80.2 ± 1.5	91.7 ± 1.7	95.8 ± 1.8	102.2 ± 1.9	99.4 ± 1.9	103.0 ± 2.0	108.6 ± 2.1	114.5 ± 2.2

ND not determined

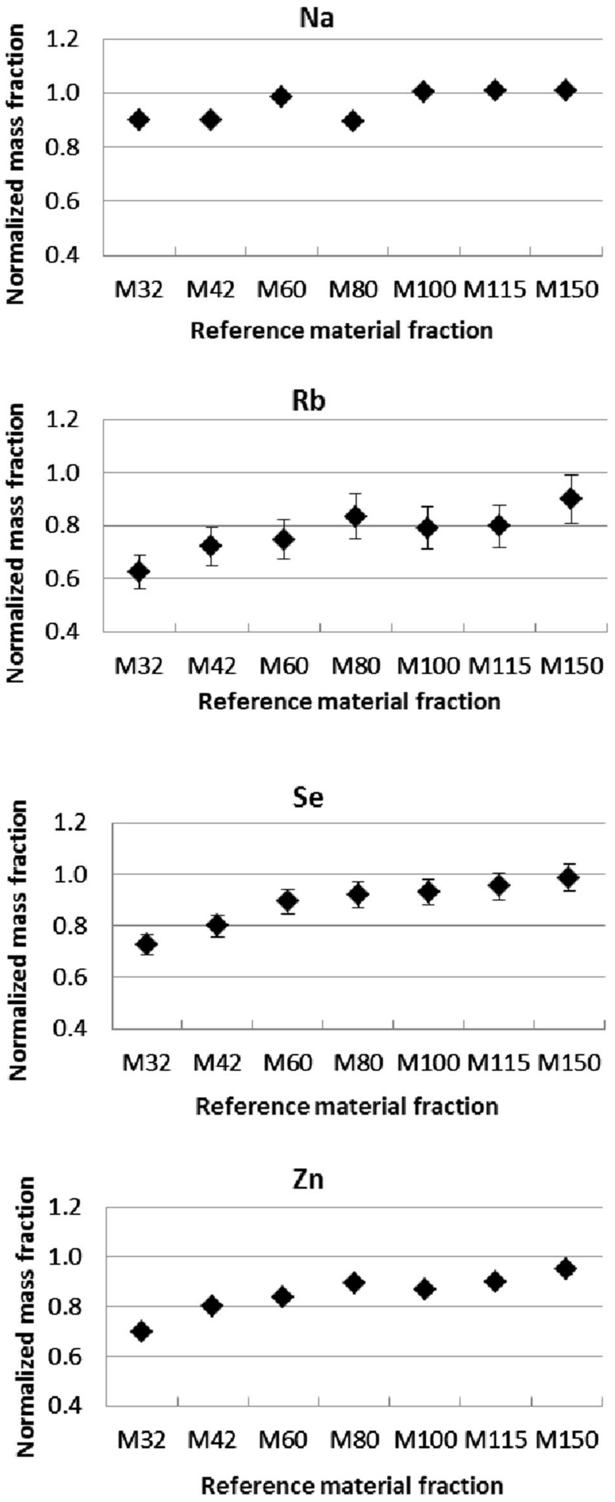

Fig. 1 Normalized mass fractions for Na, Rb, Se and Zn obtained by INAA for different fractions of the mussel candidate reference material

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, NRCC 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.

Results and discussion

Instrumental neutron activation analysis

For the purpose of quality assurance of the used INAA method, mass fractions obtained for the investigated elements in 4 certified reference materials of marine origin were compared to certified values by means of *z*-scores as presented in Table 2. The *z*-scores were calculated using the modified Horwitz equation as the criterion to estimate fit for purpose target standard deviations [12, 19–21]. Satisfactory *z*-score results were obtained for most of the elements in all the analyzed reference materials and it was concluded that the INAA method is accurate for the analysis of freeze-dried biological materials as $|z\text{-score}| \leq 2$ [19]. It was not possible to investigate the method performance for Eu and La as these elements are not certified in the analyzed reference materials. In the case of La, the element was not detected in the analyzed reference materials. Questionable *z*-score values were obtained for Br and Th in NIST SRM 2976. In the case of Br, only reference value is available in the certificate and then the obtained *z*-score should not be regarded as significant. As for Th, the obtained *z*-score may be attributed to the level of the element in the reference material, which is lower than the estimated quantification limit of the method ($L_Q = 0.038$ mg kg^{-1}).

Table 3 presents the element mass fractions obtained by INAA for Ag, As, Br, Co, Cr, Cs, Eu, Fe, La, Na, Rb, Sc, 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 Fig. 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

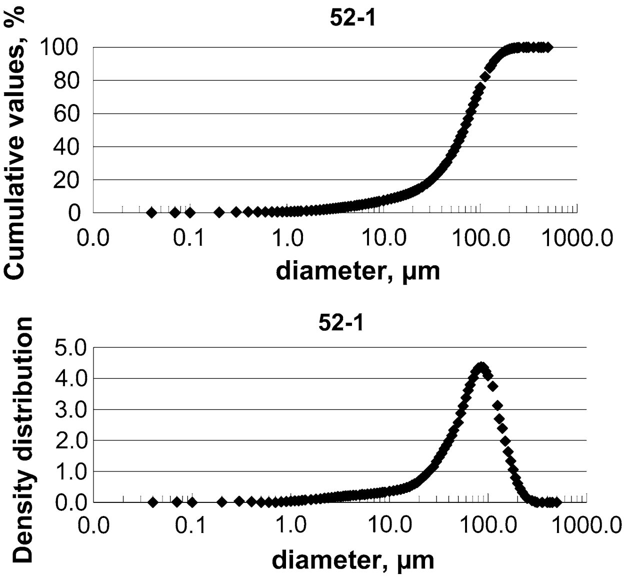

Fig. 2 Particle size distribution for bottle 52 of the mussel candidate reference material

homogeneity problems during the sample intake by end-users. The observed trend was opposite to the reported for a lichen reference material in which lower element contents were observed for reference material fractions with smaller particle sizes [12]. Besides being a different matrix material, the particle size distribution range was much lower in this study (between <20 µm and <64 µm) making difficult any comparison between the observed trends.

Particle size distribution

During the tests performed to characterize the particle size distribution of the mussel reference material by laser diffraction, it was concluded that water should 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 2 shows the typical particle size distribution profile of the mussel candidate reference material obtained in volume/undersize for bottle 52. It was observed that the mussel candidate reference material presents mean particle size of $\mu = 94.6 \pm 0.8$ µm, with Gaussian distribution (coefficient of determination $R^2 = 0.97$ for Gaussian curve fitting). 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]. The distribution profile obtained for bottles 100 and 162 perfectly overlapped with that in Fig. 2 and, hence, great similarity was observed among the particle size distributions of bottles chosen from the beginning, middle and end of the bottling process. This fact indicates that the bottling process was satisfactory, showing that there is no major differences between bottles of the material with respect to particle size, supporting the results obtained for the homogeneity study of the reference material.

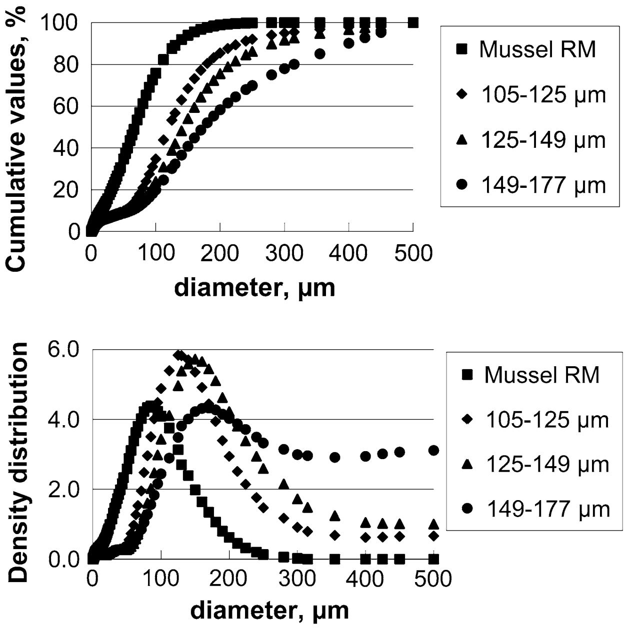

Fig. 3 Particle size distribution for different fractions of the mussel candidate reference material

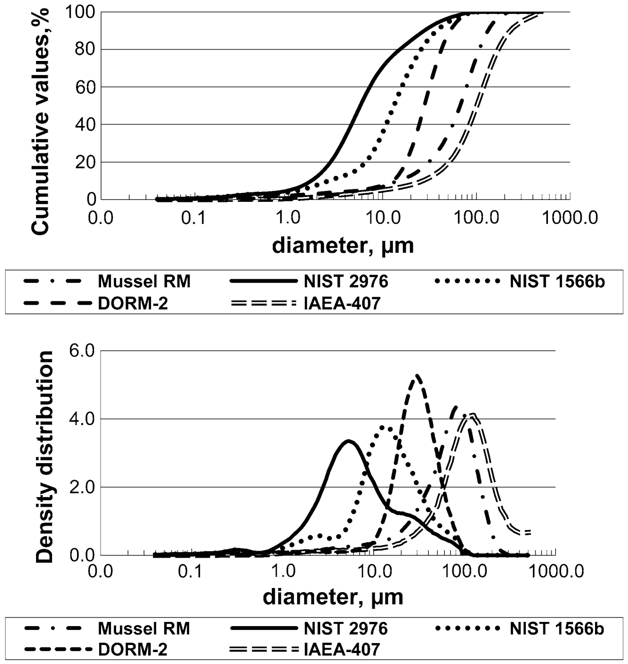

Fig. 4 Particle size distribution comparison for the mussel candidate reference material and different certified reference materials

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 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 Fig. 4. It was also noted that the other certified reference materials presented finer grain size distributions, the result of applying different milling processes in the production of these materials.

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 coarser particle size distributions, indicating the importance of particle size control in the production of biological reference materials.

Acknowledgments Authors are indebted to the financial support received from IPEN—CNEN/SP; the State of São Paulo Research Foundation (FAPESP process number 2006/06556-7) and the Brazilian National Council for Scientific and Technological Development (CNPq process number 472736/2008-6).

References

1. Zschunke A (ed) (2000) Reference materials in analytical chemistry—a guide for selection and use. Springer, Berlin
2. Kramer KJM, Kramer GN, Muntau H (2001) Practical manual for the production of laboratory reference materials. Mermayde, Bergen
3. Levoguer C, Kippax P (2011) Laser diffraction: a firm foundation for particle characterization. Am Lab 43:13–16
4. Jillavenkatesa A, Dapkunas SJ, Lum LH (2001) Particle size characterization. NIST Special Publication 960-1, NIST, Washington
5. Lai CY, Groth A, Gray S, Duke M (2014) Preparation and characterization of poly(vinylidene fluoride)/nanoclay nanocomposite flat sheet membranes for abrasion resistance. Water Res 57:56–66
6. Gmoshinski IV, Khotimchenko SA, Popov VO, Dzantiev BB, Zherdev AV, Demin VF, Buzulukov YP (2013) Nanomaterials and nanotechnologies: methods of analysis and control. Russ Chem Rev 82(1):48–76
7. Handy RD, Owen R, Valsami-Jones E (2008) The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. Ecotoxicology 17(5):315–325
8. Hamilton TF, Jernströem J, Martinelli RE, Kehl SR, Eriksson M, Williams RW, Bielewski M, Rivers AN, Brown TA, Tumey SJ, Betti M (2009) Frequency distribution, isotopic composition and physical characterization of plutonium-bearing particles from the Fig-Quince zone on Runit Island, Enewetak Atoll. J Radioanal Nucl Chem 282(3):1019–1026
9. Rizzio E, Bergamaschi G, Profumo A, Gallorini M (2001) The use of neutron activation analysis for particle size fractionation and chemical characterization of trace elements in urban air particulate matter. J Radioanal Nucl Chem 248(1):21–28
10. Mitkus RJ, Powell JL, Zeisler R, Squibb KS (2013) Comparative physicochemical and biological characterization of NIST interim reference material $PM_{2.5}$ and SRM 1648 in human A549 and mouse RAW264.7 cells. Toxicol In Vitro 27:2289–2298
11. Donghui H, Hongchao S, Bangfa N, Weizhi T, Yuanxun Z, Pingsheng W, Xiong LC, Guiying Z, Caijin X, Haiqing Z, Changjun Z (2011) Characterization of sampling behavior for multielements in NIST SRM 2703. J Radioanal Nucl Chem 288(1):163–169
12. Marques AP, Freitas MC, Wolterbeek HT, Verburg TG, de Goeij JJM (2007) Grain-size effects on PIXE and INAA analysis of IAEA-336 lichen reference material. Nucl Instr Meth Phys Res B 255:380–394
13. Ulrich T, Kamber BS (2013) Natural obsidian glass as an external accuracy reference material in laser ablation-inductively coupled plasma-mass spectrometry. Geostand Geoanal Res 37(2):169–188
14. Santos D Jr, Nunes LC, Carvalho GGA, Gomes MS, Souza PF, Leme FO, Santos LGC, Krug FJ (2012) Laser-induced breakdown spectroscopy for analysis of plant materials: a review. Spectrochim Acta B 71–72:3–13
15. Pasquini C, Cortez J, Silva LMC, Gonzaga FB (2007) Laser induced breakdown spectroscopy. J Braz Chem Soc 18(3):463–512
16. Moreira EG, Vasconcellos MBA, Maihara VA, Catharino MGM, Saiki M (2007) Mussel reference material preparation proposal as a quality assurance tool for Brazilian seashore biomonitoring. J Braz Soc Ecotoxicol 2: 61–65. http://ecotoxbrasil.org.br. Accessed 10 Jul 2014
17. Moreira EG (2010) Preparo e caracterização de um material de referência de mexilhão *Perna perna* (Linnaeus, 1758). Doctoral Thesis, São Paulo University, São Paulo. http://www.teses.usp.br/teses/disponiveis/85/85131/tde-21062011-093245/en.php. Accessed 10 Jul 2014
18. Moreira EG, Vasconcellos MBA, Saiki M (2006) Uncertainty assessment in instrumental neutron activation analysis of biological materials. J Radioanal Nucl Chem 269:377–382
19. Prichard E, Barwick V (2008) Quality assurance in analytical chemistry. Wiley-LGC, Teddington
20. Thompson M (2000) Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. Analyst 125:385–386
21. Moreira EG, Seo D, Vasconcellos MBA, Saiki M (2013) Trace element determination in a mussel reference material using short irradiation instrumental neutron activation analysis. J Radioanal Nucl Chem 296:251–254