



ICMGP 2024
CAPE TOWN • SOUTH AFRICA • 21 - 26 JULY

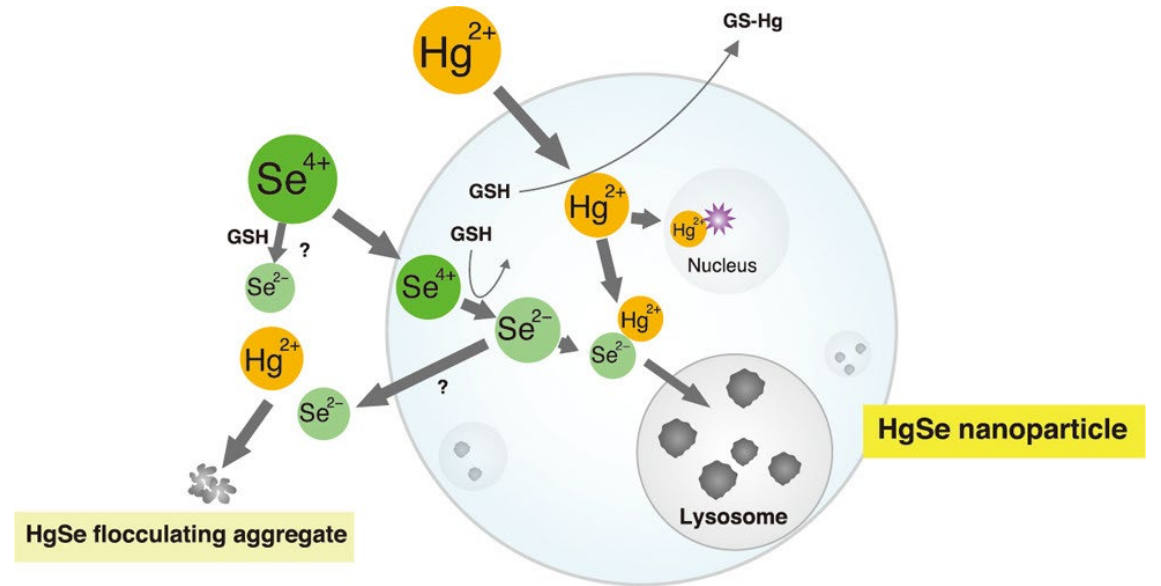
Critical assessment of sample preparation methods for the study of HgSe nanoparticles in fish tissues by SP-ICP-MS

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R.C. Rodríguez Martín-Doimeadios**

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University of Castilla-La Mancha

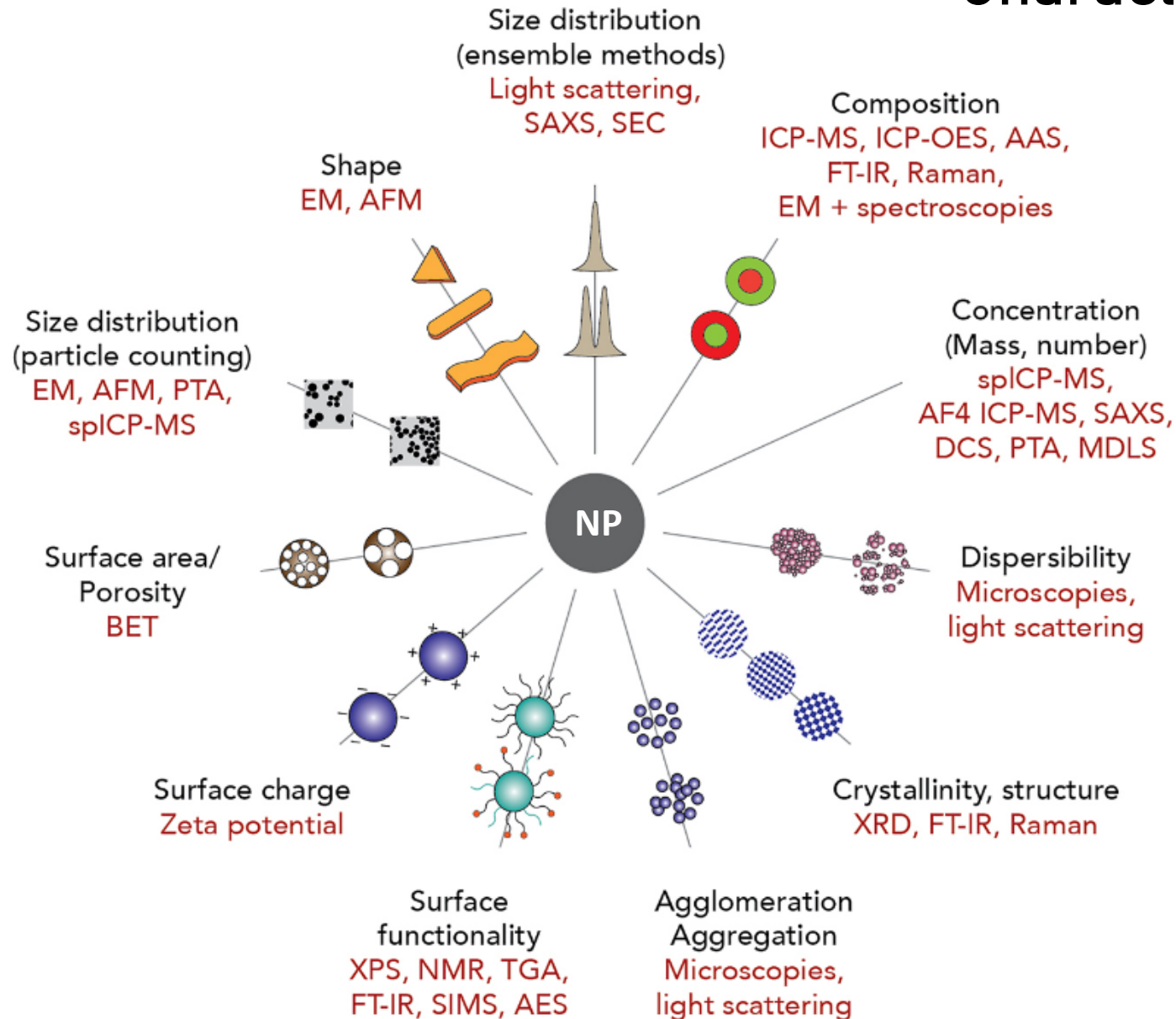
E-mail: rosacarmen.rodriguez@uclm.es



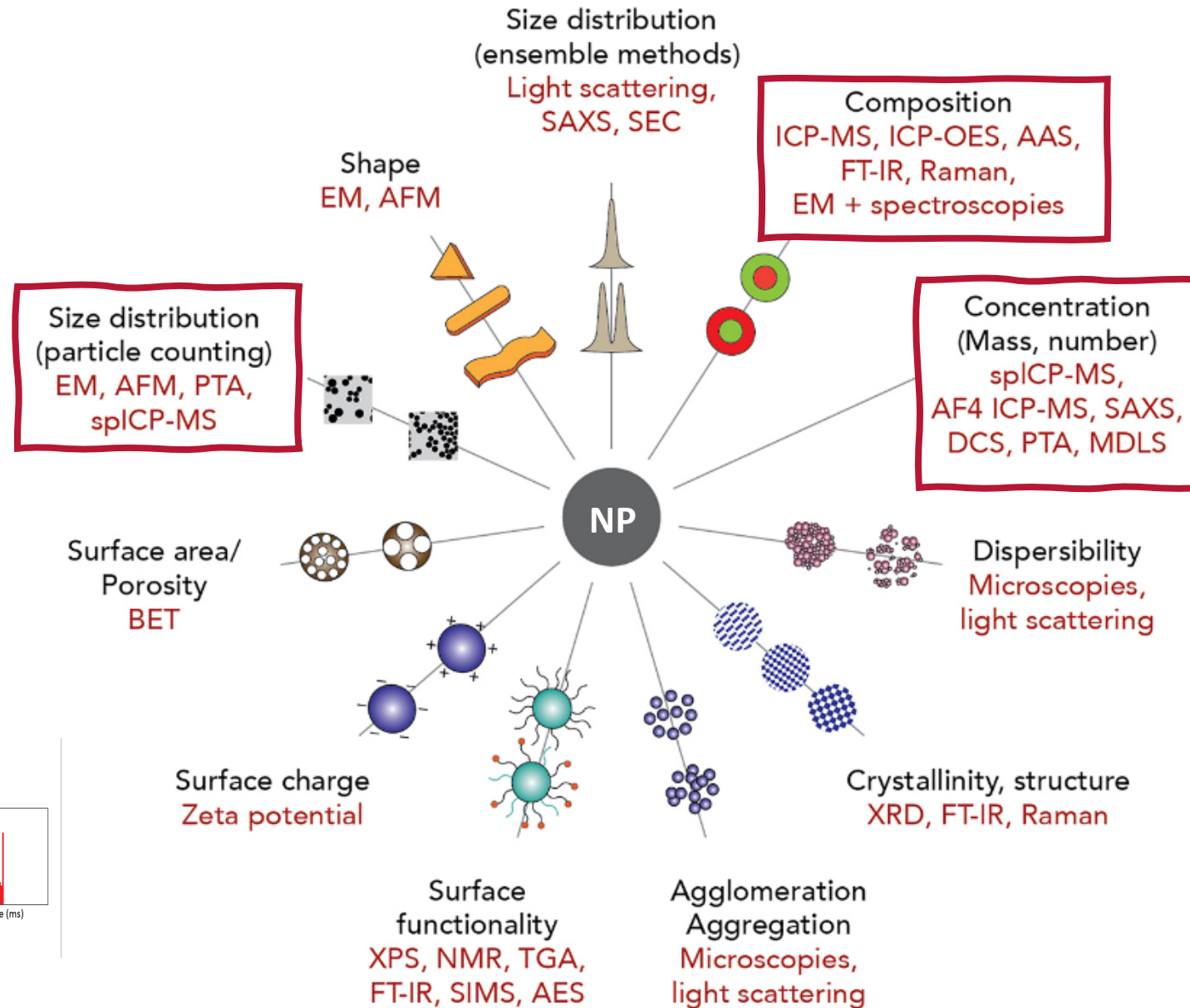


Tanaka et al., Chemical Research in Toxicology (2021)

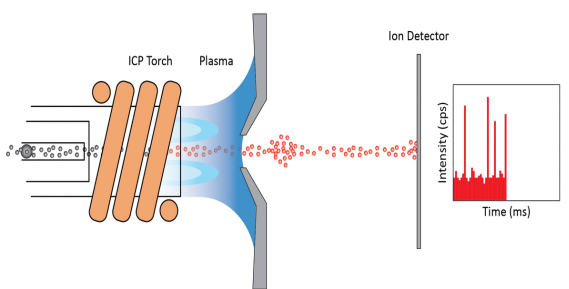
Characterization of NPs



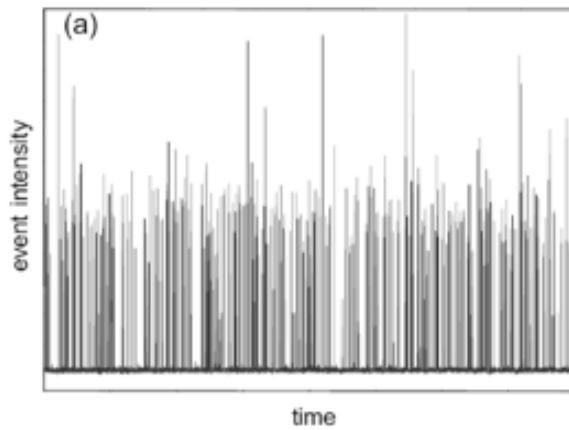
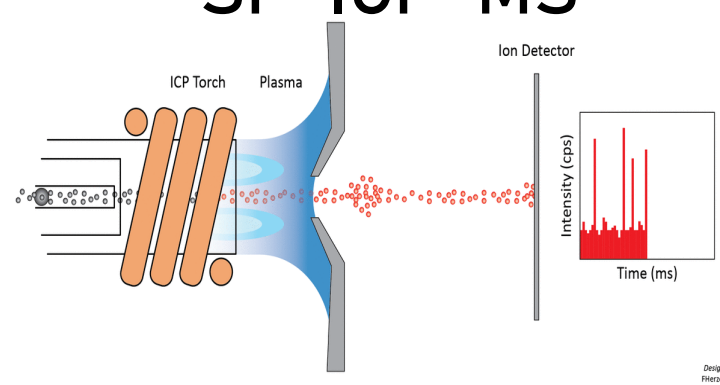
Characterization of NPs



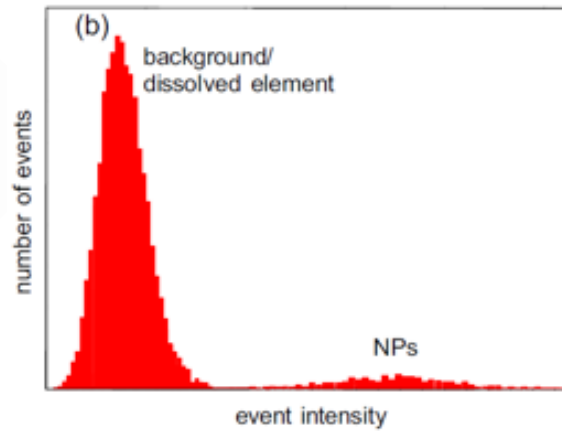
Single Particle-ICP-MS



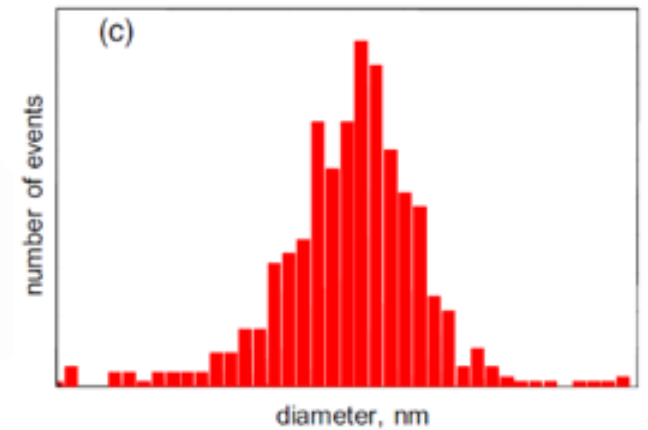
SP-ICP-MS



Data acquisition

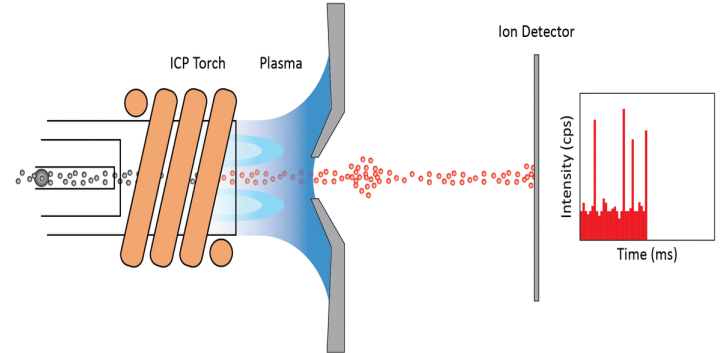


Data treatment



Samples must be liquid and properly diluted!

SP-ICP-MS



Sample preparation

- *Non-quantitative recoveries*
- *Possible dissolution and/or artificial formation of NPs*

Sample preparation: HgSeNPs & SP-ICP-MS

$\nu \pi \dagger \ll \pi \cdot \check{s},$

- Enzymes
- Formic acid
- ...

$\delta \cdot \pi^- \ll \check{G}$

- Sonication (bath/probe)
- Heating-block
- Heat-shaking
- ...

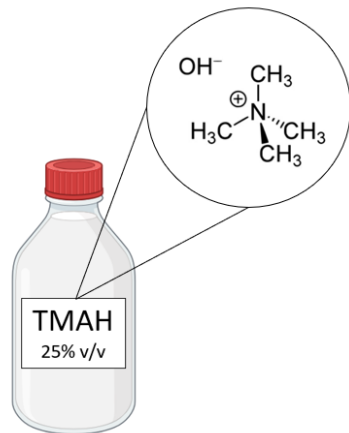
$z \check{s} \pi_1 ,$

- Defatting
- Incubation with reagents
- Application of a source of energy
- Ultracentrifugation
- ...

Sample preparation: HgSeNPs & SP-ICP-MS

∇ π † « π · Š,

- Enzymes
- Formic acid
- ...



TETRAMETHYLAMMONIUM
HYDROXIDE

8 · π⁻ « Ğ

- Sonication (bath/probe)
- Heating-block
- Heat-shaking
- ...



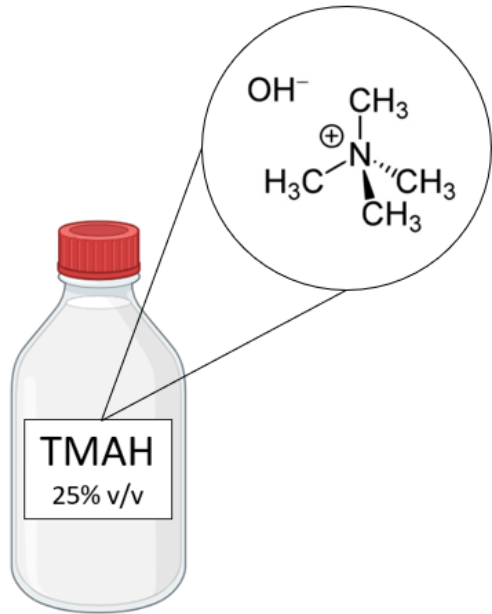
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Z Šπ1 ,

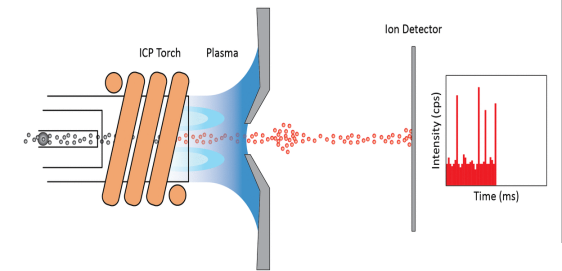
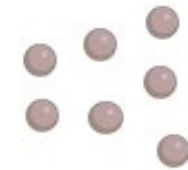
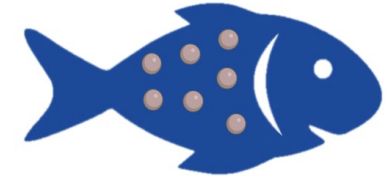
- Defatting
- Incubation with reagents
- Application of a source of energy
- Ultracentrifugation
- ...

Objective

TETRAMETHYLAMMONIUM
HYDROXIDE



ETHOS PLUS



Critical evaluation

z s 7Ms 7_z

2 †š† š̄ π†š† π· š

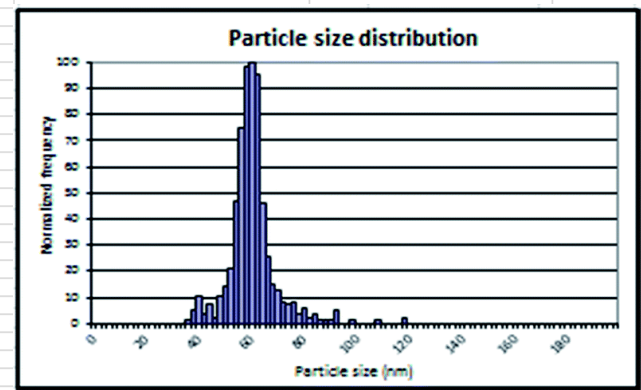
	A	B	D	E	F	G	H	I
1	IPUT (paste special; value)	Sample	Mezcla	Mezcla	Mezcla	Mass (fg)	Mass (fg)	Diámetro (nm)
2	dwell time event	202Hg	Background 202Hg	NPs 202Hg	NPs 202Hg			
3	1	6801.850103	6802		0	0		
4	2	6201.537981	6202		0	0		
5	3	5601.254681	5601		0	0		
6	4	3800.577688	3801		0	0		
7	5	7902.43436		2672	2672	0.456818676	0.45681868	47.19424528
8	6	6401.63882	6402		0	0		
9	7	6601.74286	6602		0	0		
10	8	3600.518475	3601		0	0		
11	9	5001.0002	5001		0	0		
12	10	5001.0002	5001		0	0		
13	11	6801.850103	6802		0	0		
14	12	6401.63882	6402		0	0		
15	13	5401.166652	5401		0	0		
16	14	7001.360549	7002		0	0		
17	15	9803.098691		3673	3673	0.627879241	0.62787924	52.47266679
18	16	6201.537981	6202		0	0		
19	17	4200.705719	4201		0	0		
20	18	4200.705719	4201		0	0		
21	19	3600.518475	3601		0	0		
22	20	4600.846556	4601		0	0		
23	21	4400.774536	4401		0	0		
24	22	6801.850103	6802		0	0		
25	23	4800.921777	4801		0	0		
26	24	4400.774536	4401		0	0		
27	25	5001.0002	5001		0	0		
28	26	4400.774536	4401		0	0		
29	27	5001.0002	5001		0	0		
30	28	5401.166652	5401		0	0		
31	29	6001.440346	6001		0	0		
32	30	3600.518475	3601		0	0		
33	31	6001.440346	6001		0	0		
34	32	6401.63882	6402		0	0		
35	33	6601.74286	6602		0	0		
36	34	6401.63882	6402		0	0		
37	35	4800.921777	4801		0	0		
38	36	6801.850103	6802		0	0		
39	37	6601.74286	6602		0	0		
40	38	6801.850103	6802		0	0		
41	39	8402.823349		3273	3273	0.559453372	0.55945337	50.49275419
42	40	7802.43436		2672	2672	0.456818676	0.45681868	47.19424528
43	41	7001.360549	7002		0	0		
44	42	7202.074197		2072	2072	0.354188908	0.35418891	43.35635335
45	43	2800.313635	2800		0	0		
46	44	5001.0002	5001		0	0		
47	45	4400.774536	4401		0	0		
48	46	3200.409652	3200		0	0		
49	47	2800.313635	2800		0	0		
50	48	4600.846556	4601		0	0		
51	49	3000.360043	3000		0	0		
52	50	5601.254681	5601		0	0		
53	51	5801.345912	5801		0	0		
54	52	5001.0002	5001		0	0		
55	53	6801.850103	6802		0	0		
56	54	6001.440346	6001		0	0		
57	55	18012.96934		12883	12883	2.202278995	2.202279	79.72573664

L	M	N	O	R	S	T
Threshold	7078.238274					
Promedio Background	5130					
Conteo	5039					
Total fg	22924.40093					
Composition	HgSe					
Molar mass particle	0.2825					
ICP-MS sensitivity	3794	cps/(µg/L)				
Dwell time	5	ms				
TE	0.095					
Densidad (g/ml)	8.3	g/mL				
Sample flow rate	0.29	mL/min				

Concentration results		
Particle concentration (number-based)	18290381.13	particles/L
Particle concentration (mass-based)	832.1016671	ng/L
Concentration p/mL	18290.38113	p/mL

Diameter results		
Promedio Diámetro	58.14835108	nm
Desviación diámetro	4.139468323	nm

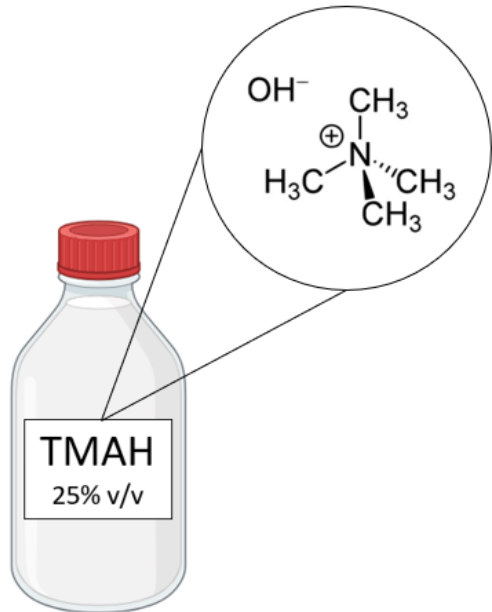
Contar background	25167	
Promedio background	5130.81841	
desviacion background	1097.245056	
Ionic concentration	1352.350662	ng/L



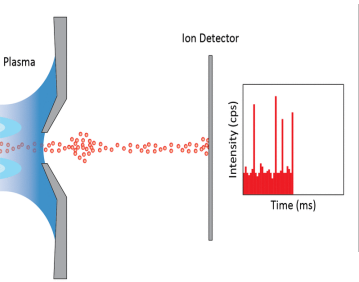
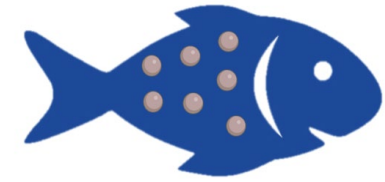
Single Particle Calculation tool (SPC)

Optimization

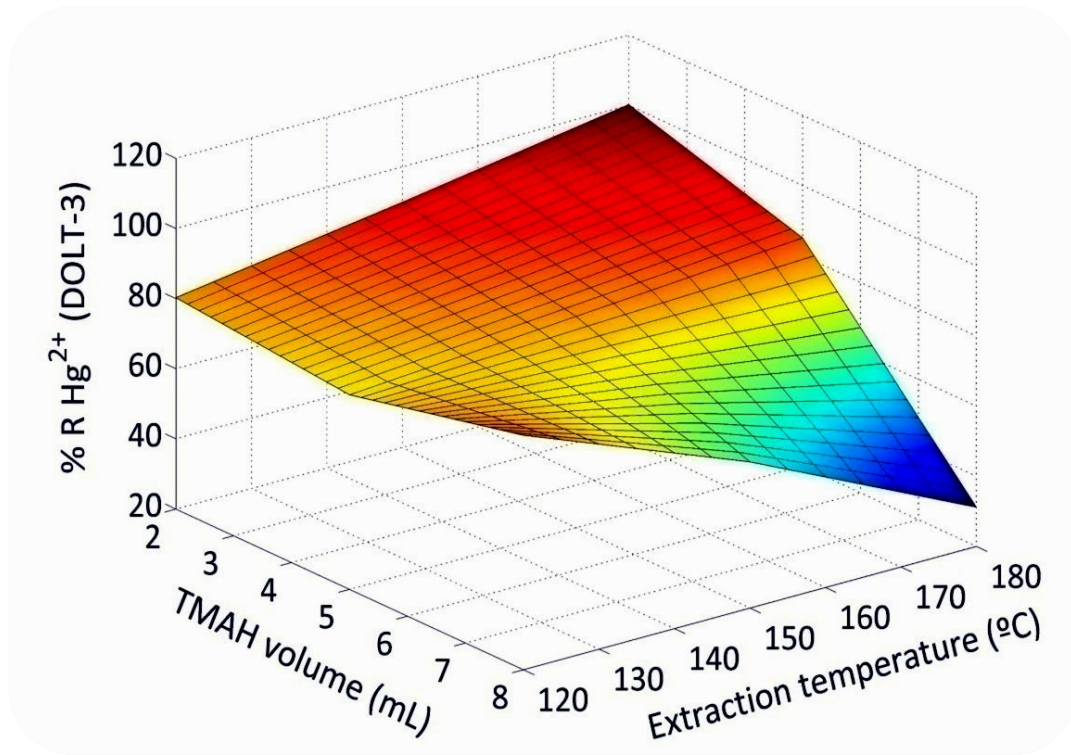
TETRAMETHYLAMMONIUM
HYDROXIDE



_Mvfï i8



zs 7Ms 7_z



**Berzas-Nevado et al., Analytical letters (2006)*

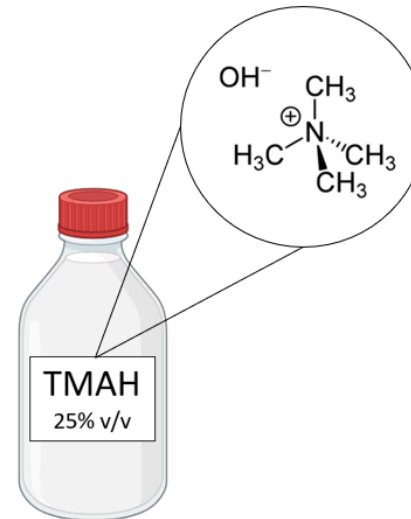
π⁻ ΑΕΓ, 1 π ΑΕΣΑ: Ε ΑΕΑ šΕ, Δπ, Γ

Ç ‡ † F⁻ - : ÇΕ π ‡ Ε:

_ i ε β Γ

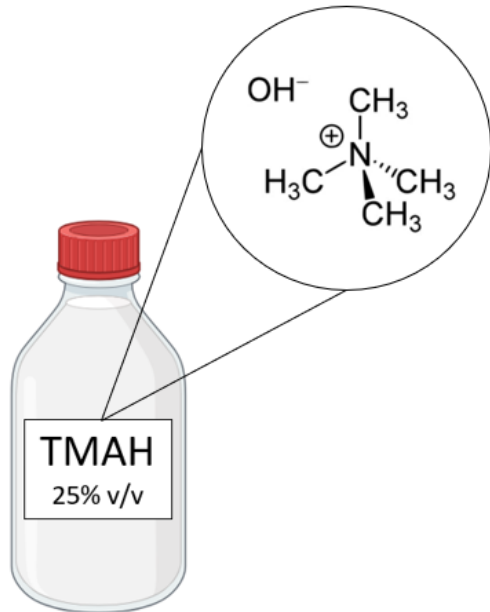
180

10+10



Optimization

TETRAMETHYLAMMONIUM
HYDROXIDE



_Mvf i 8



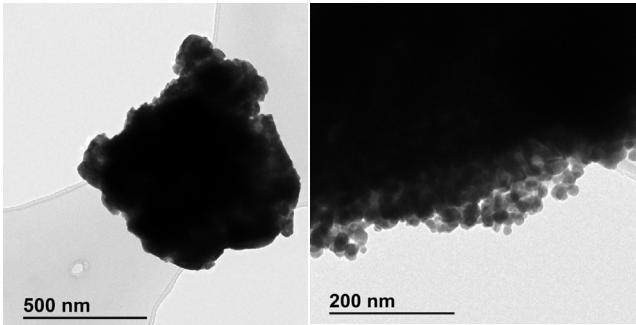
Commercial HgSe*
spike on real fish
samples

*Mercury selenide ≥99.99% (Hg basis), PURATREM

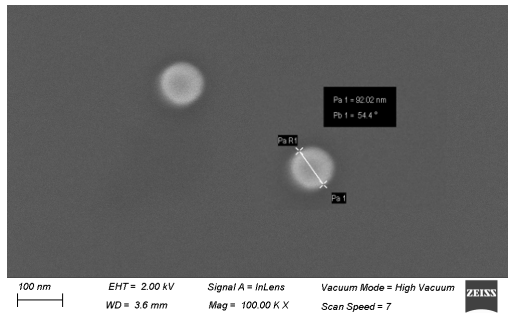
Commercial HgSe characterization



Q8_

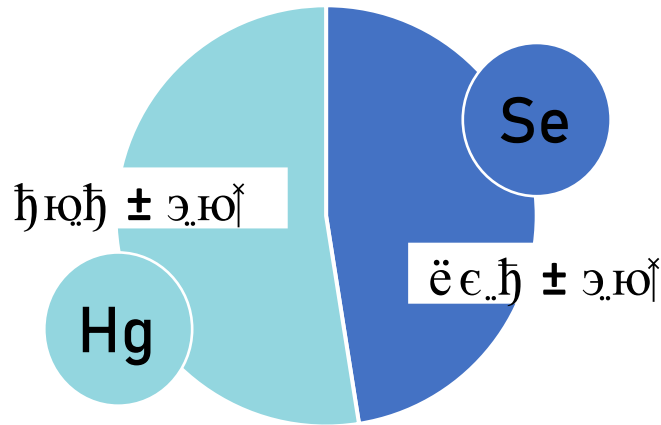
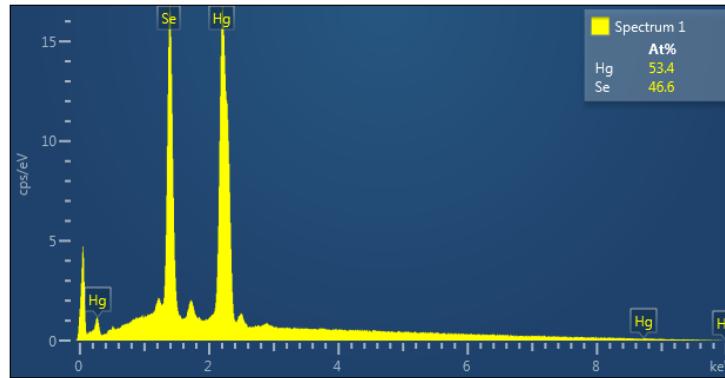


z8_



i e . r . †

82ñ

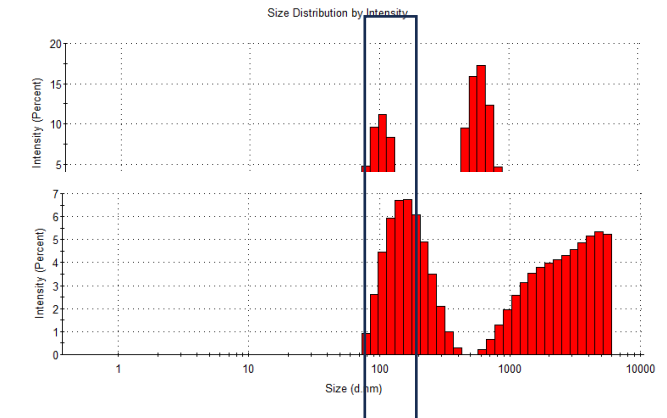


2 Zz

z A e / π · š s A G > C e i π ⁻ , G G M > π F

TMAH	0.829 ± 0.198
H ₂ O	0.682 ± 0.249

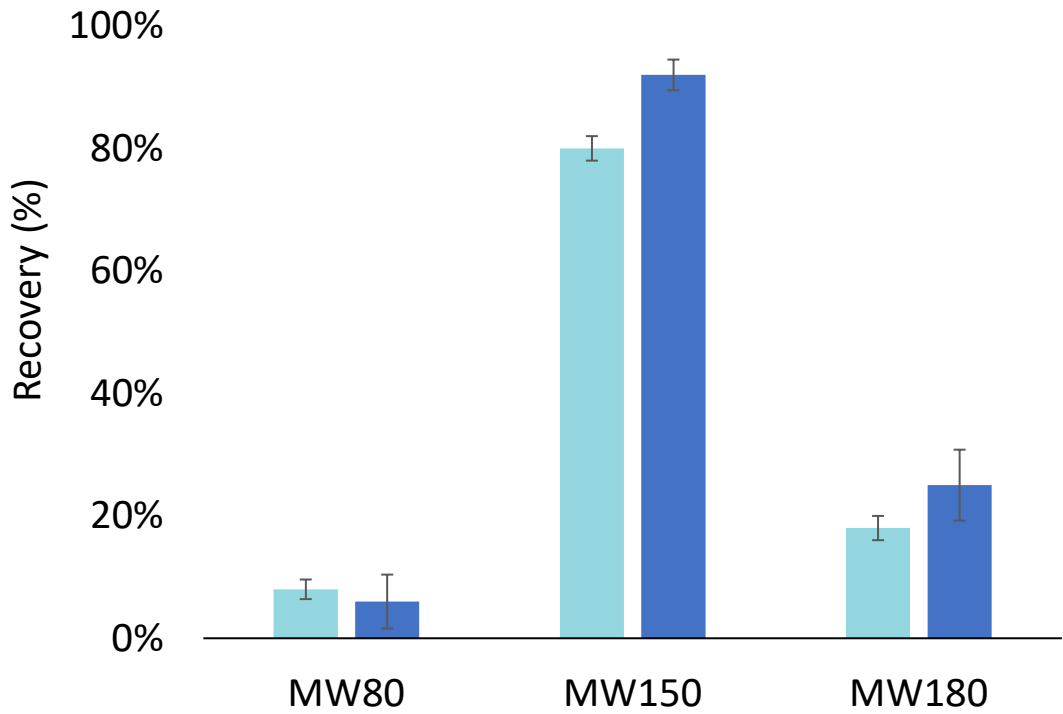
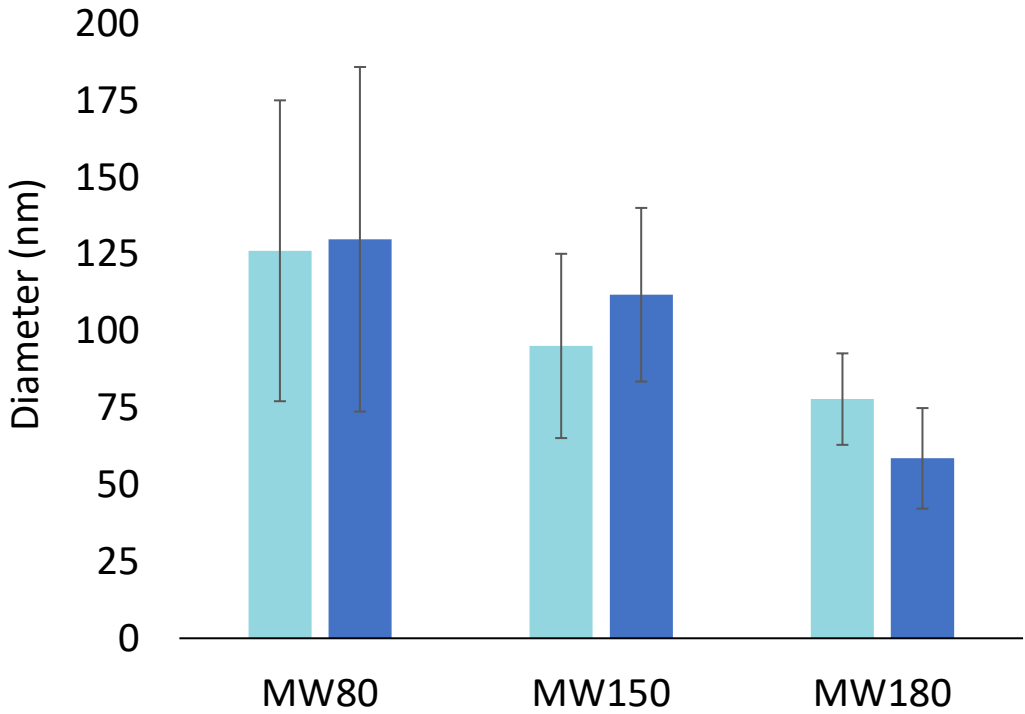
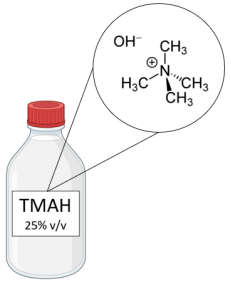
105.7 nm



Highly polydisperse

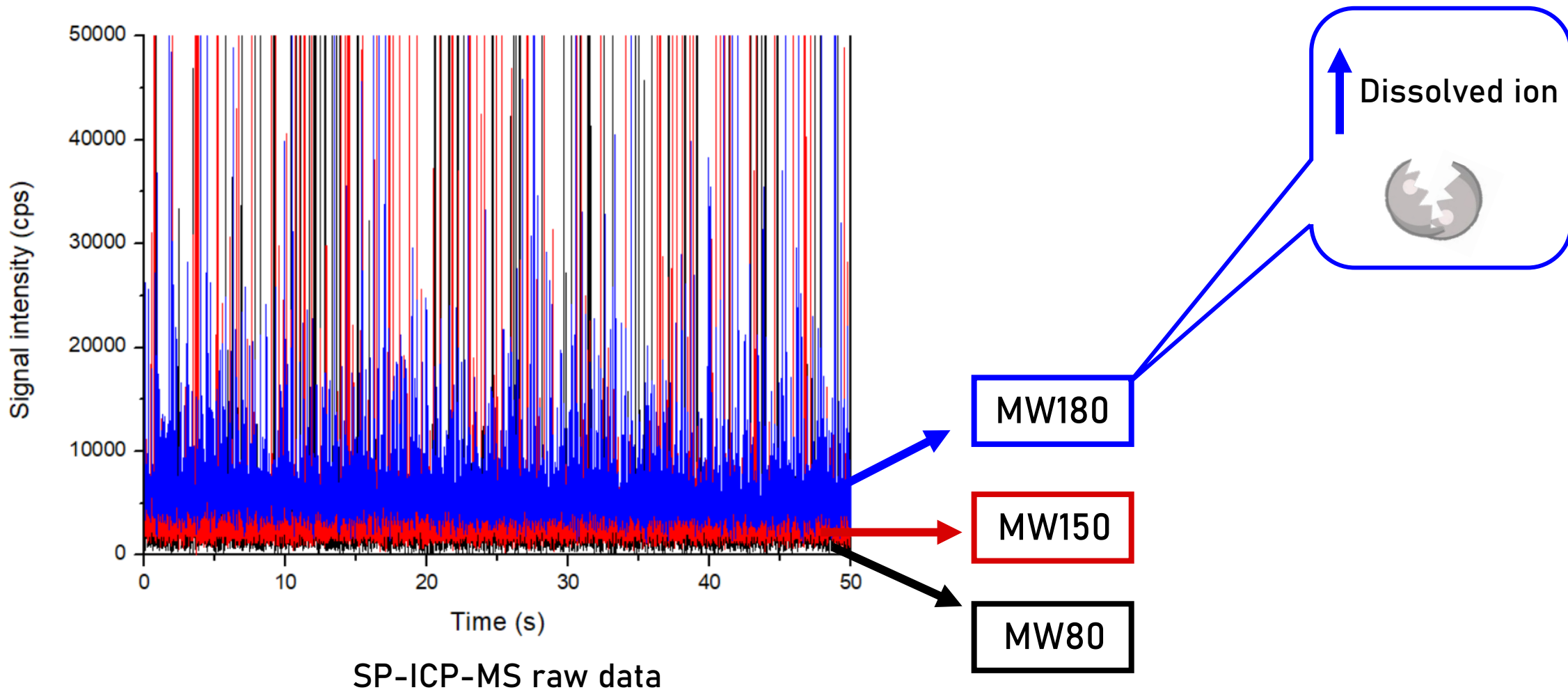
Commercial HgSe

SP-ICP-MS





■ Hg ■ Se

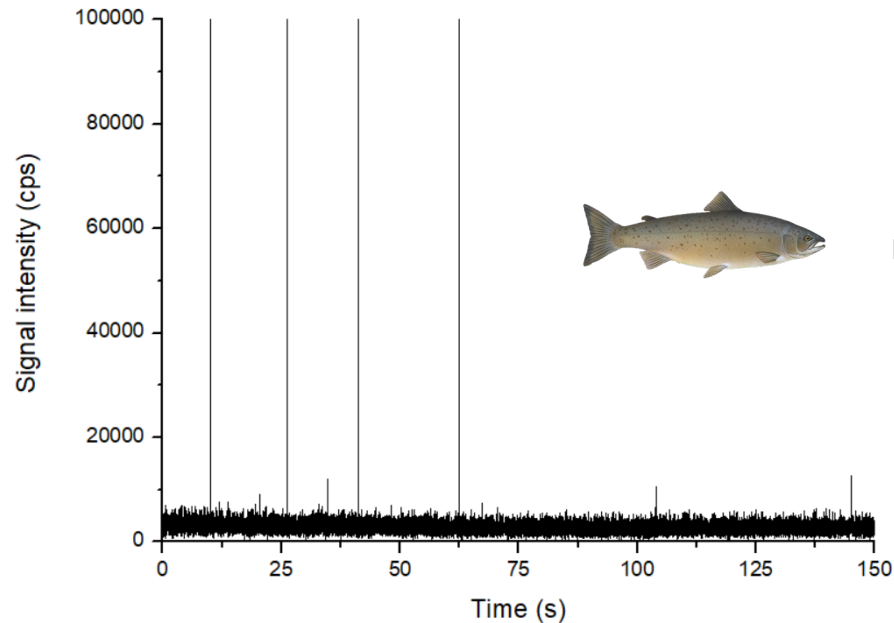
Commercial HgSe

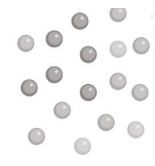


- $\dot{A} \ddot{\pi} \pi^{-} \text{ACEK} \ll z \pi, 1 \text{G} \pi \check{A} \Delta \text{E} \tilde{A}^{-} \pi \dagger \text{E}, \ddot{\pi} 1 \text{E} \pi,$

		Tissue	n	Hg (mg kg ⁻¹)	Se (mg kg ⁻¹)	Se:Hg molar ratio
Salmon		Muscle	3	0.2270 ± 0.0040	1.42 ± 0.14	15.9
Blue whiting		Muscle	3	0.431 ± 0.047	1.472 ± 0.061	8.7

SP-ICP-MS analysis



Low naturally occurring NPs were observed 

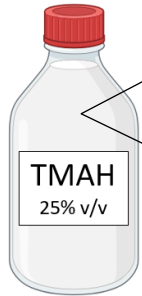
- Á† ‡ π⁻ ACCEK«z π , 1 Qπ šÁ ΔCEÃ⁻ π†€ , ‡† 1 €π,



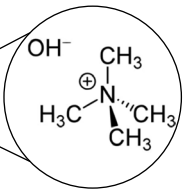
Commercial HgSe



5 mg



2 mL



0.2 g

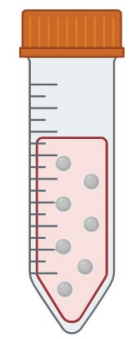
Freeze-dried fish muscle



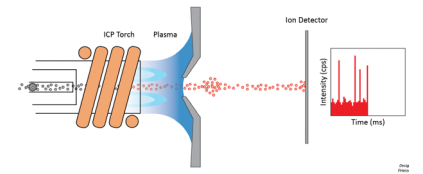
8 mL H₂O



Microwave



Dilution



z s 7Ms 7_z

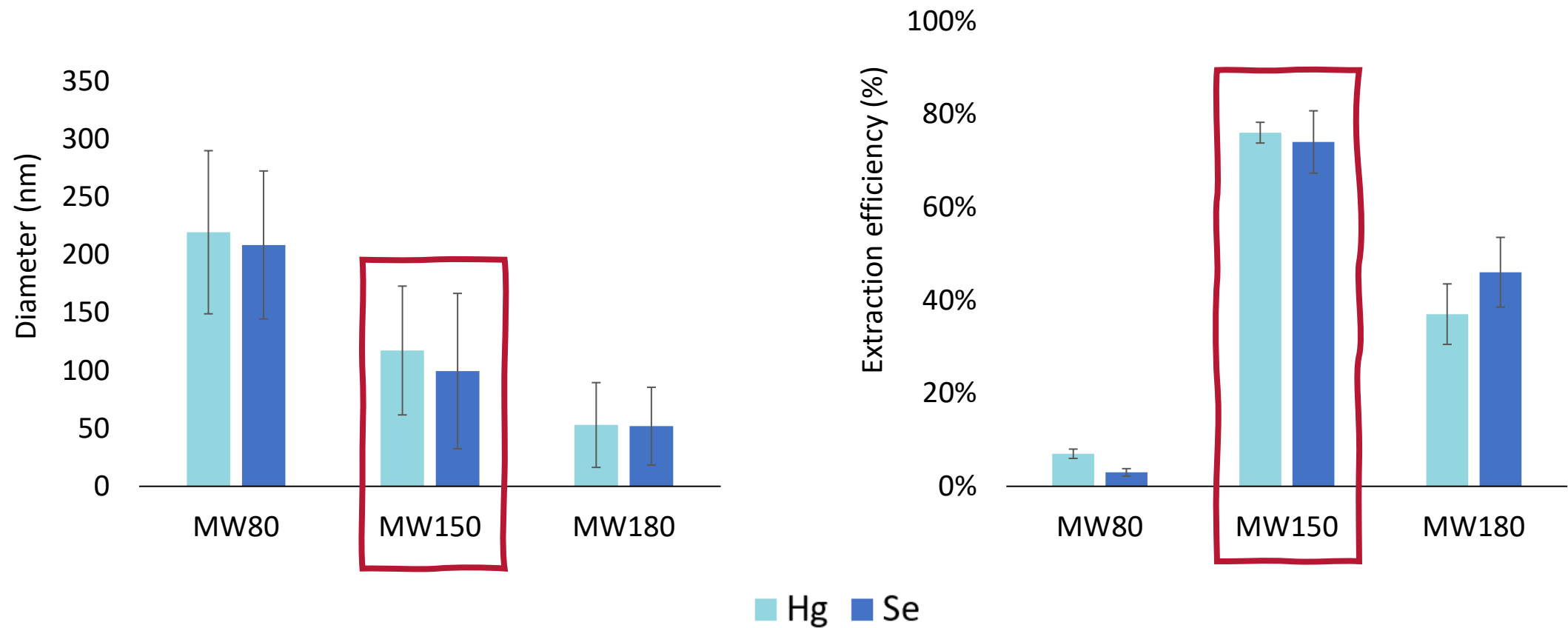
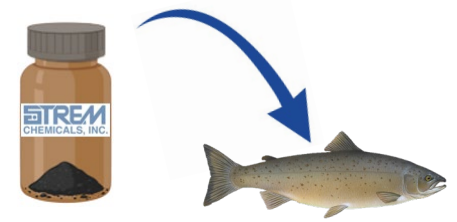
z†€ Á·



+ Qπ ¾ÃCCE«



Commercial HgSe spike on real samples

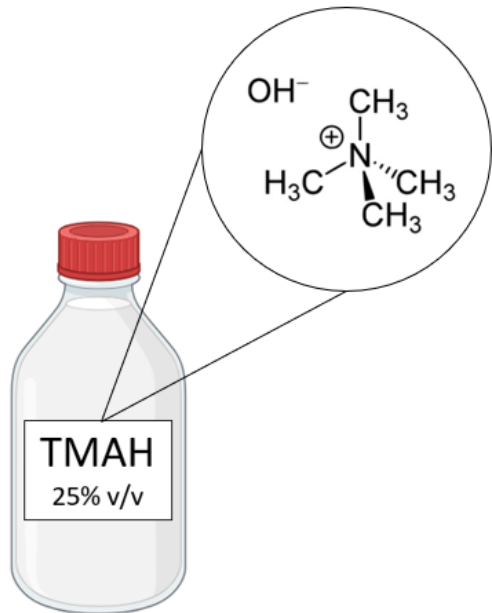


Analytical characterization

Hg	
Instrumental LOD size (nm)	36.6
Instrumental LOD concentration (particles·g ⁻¹)	4.9E+07
Procedural LOD size (nm)	43.8
Procedural LOD concentration (particles·g ⁻¹)	8.4E+07
Size % RSD intraday (n=4)	5.7
Concentration % RSD intraday (n=4)	4.0

Se	
LOD size Se (nm)	35.4
LOD concentration Se (particles·g ⁻¹)	1.8E+07
Procedural LOD size (nm)	50.8
Procedural LOD concentration (particles·g ⁻¹)	5.1E+07
Size % RSD intraday (n=4)	5.7
Concentration % RSD intraday (n=4)	2.6

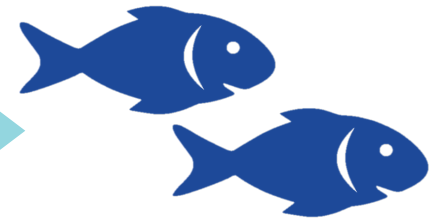
TETRAMETHYLAMMONIUM
HYDROXIDE



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Reference
materials





Real
samples

Application to reference materials



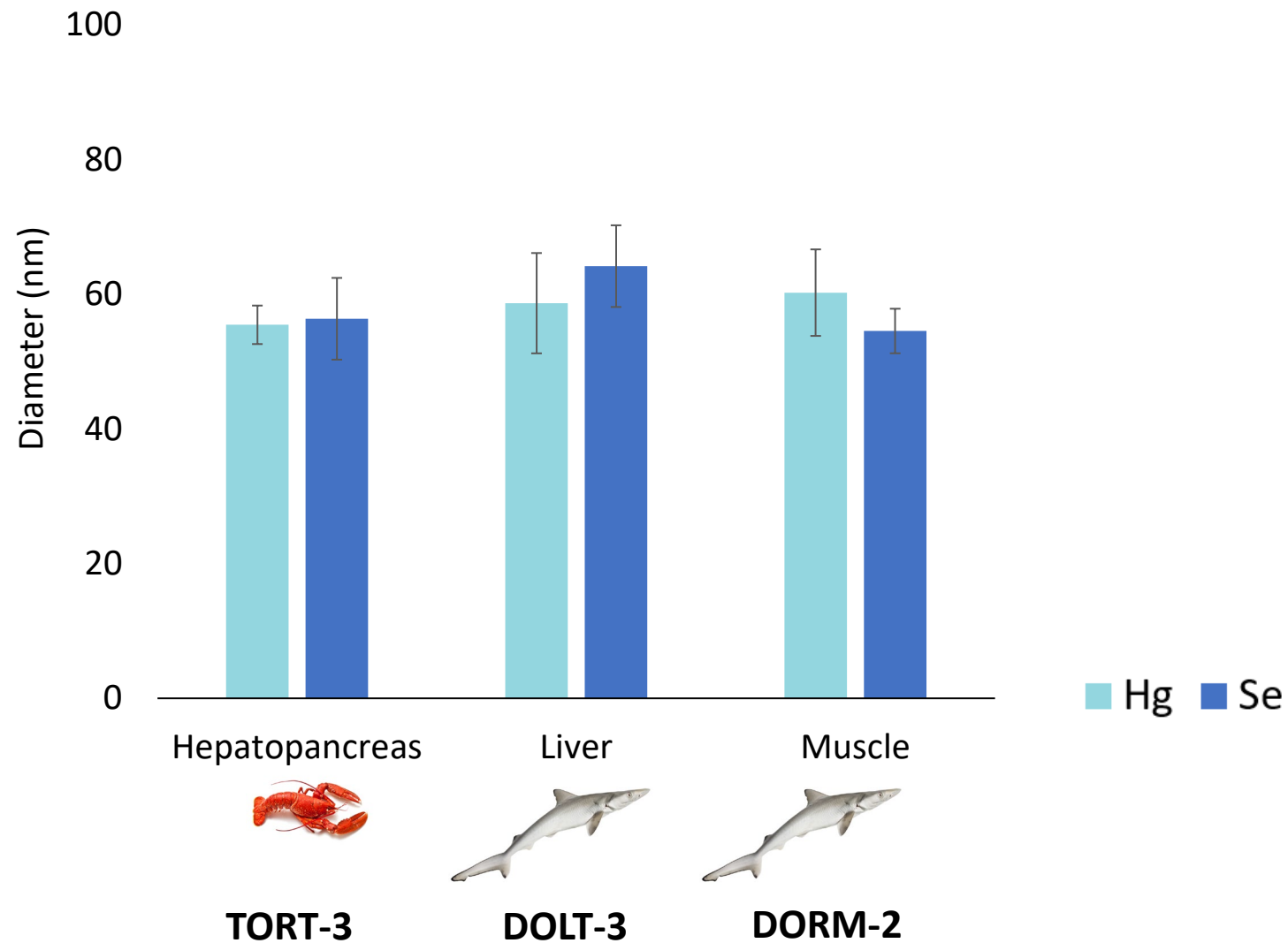
National Research
Council Canada

ICP analysis		Tissue	n	Hg (mg kg ⁻¹)	Se (mg kg ⁻¹)	Se:Hg molar ratio
TORT-3		Lobster hepatopancreas	3	0.32 ± 0.17	11.16 ± 0.43	88.6
DOLT-3		Dogfish liver	3	3.38 ± 0.22	7.17 ± 0.25	5.4
DORM-2		Dogfish muscle	3	4.77 ± 0.19	1.41 ± 0.14	0.8

Application to reference materials



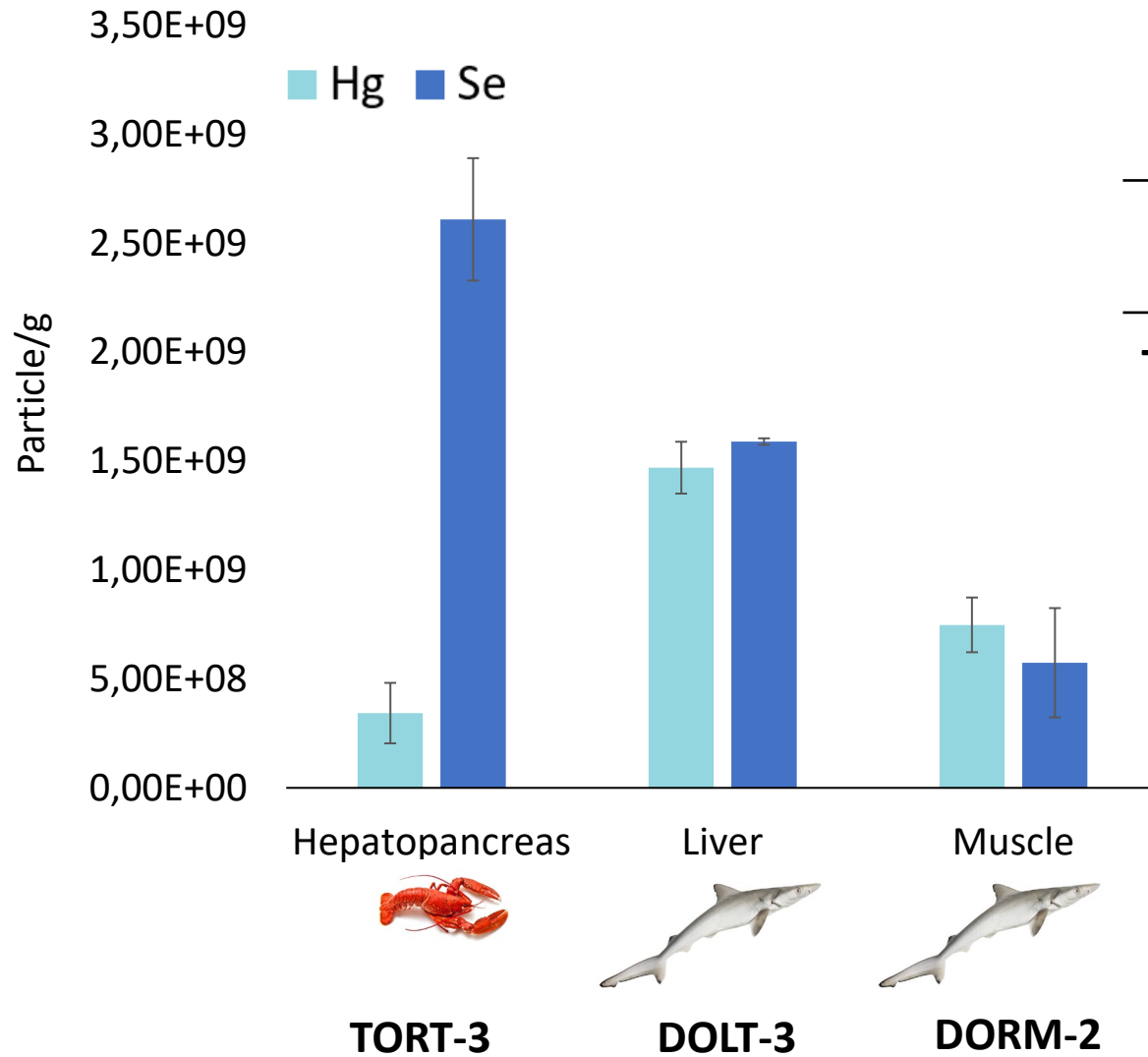
National Research
Council Canada



Application to reference materials

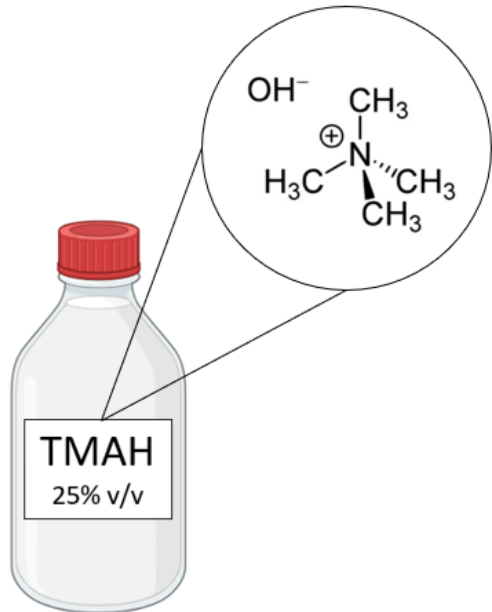


National Research Council Canada



	n	Hg (mg kg ⁻¹)	Se (mg kg ⁻¹)	Se:Hg molar ratio
TORT-3	3	0.32 ± 0.17	11.16 ± 0.43	88.6

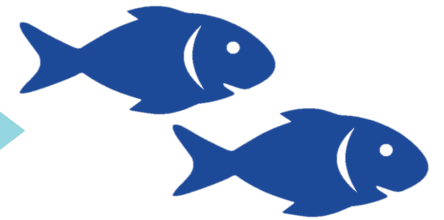
TETRAMETHYLAMMONIUM
HYDROXIDE



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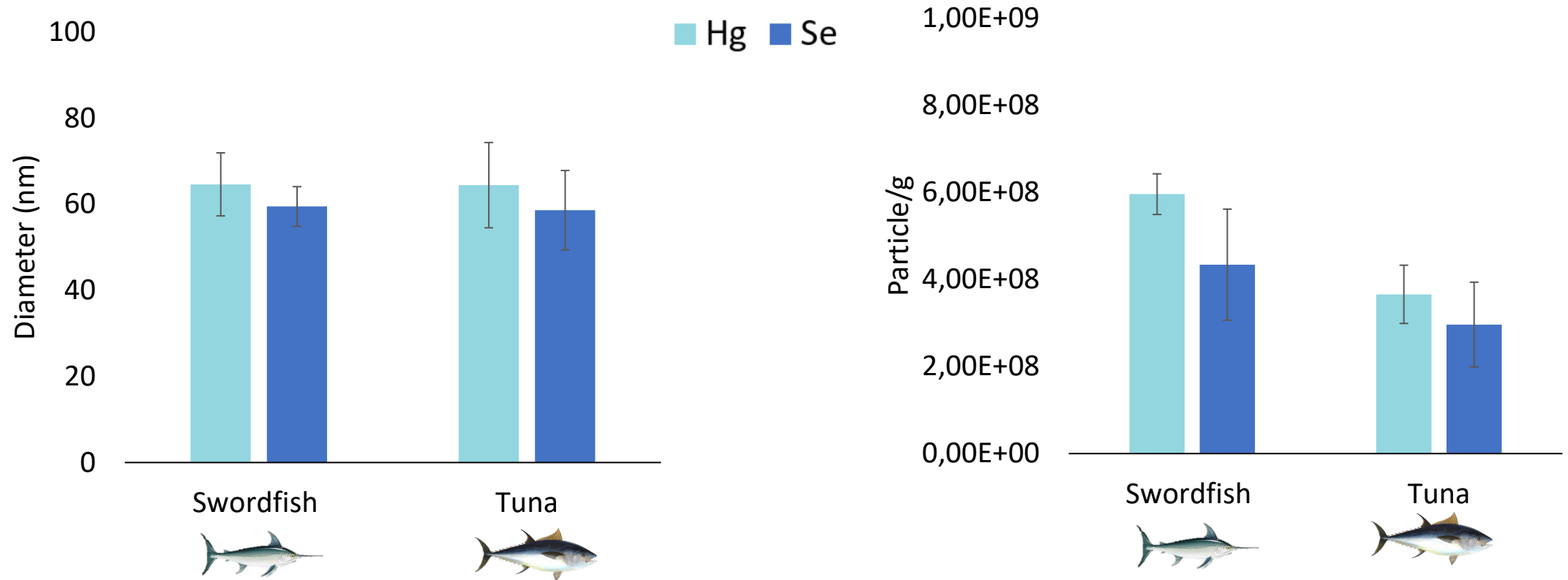
Reference
materials



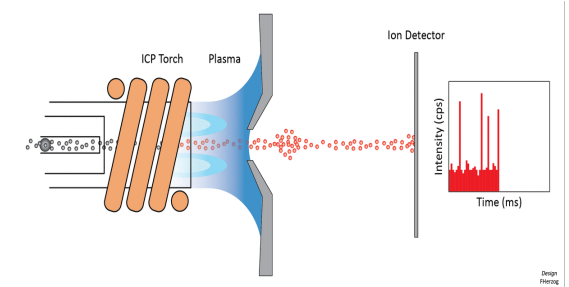
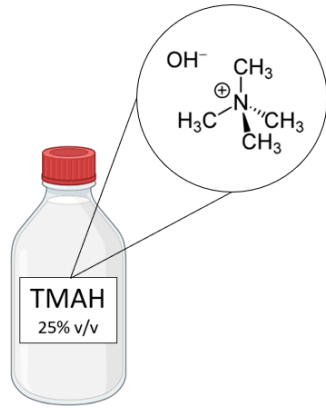
Real
samples

Application to real samples

		Tissue	n	Hg (mg kg ⁻¹)	Se (mg kg ⁻¹)	Se:Hg molar ratio
Swordfish		Muscle	3	2.66 ± 0.22	3.34 ± 0.13	3.1
Tuna		Muscle	3	2.39 ± 0.11	3.38 ± 0.14	3.6



Conclusions



✓ *Reagent for classical Hg speciation in biological matrices*

Low cost reagent

High purity (low blanks)

✓ *More energy in less time*

No pre-treatment or cleaning

Total extraction time: 20 min

High productivity

✓ *Particle number & Mass-based concentration*

Size (core)

Dissolved element

Simple, cost-effective and green procedure for the characterization and quantification of HgSeNPs in biological samples

Sample preparation: HgSeNPs & SP-ICP-MS

Reagents

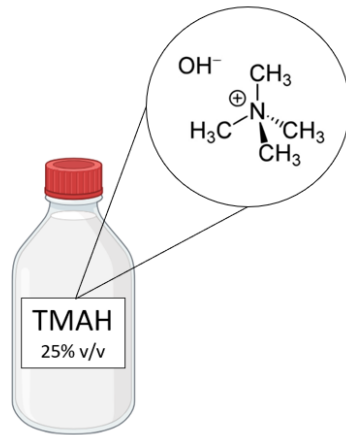
- Enzymes
- Formic acid
- ...

Energy

- Sonication (bath/probe)
- Heating-block
- Heat-shaking
- ...

Steps

- Defatting
- Incubation with reagents
- Application a source of energy
- Ultracentrifugation
- ...



TETRAMETHYLAMMONIUM
HYDROXIDE

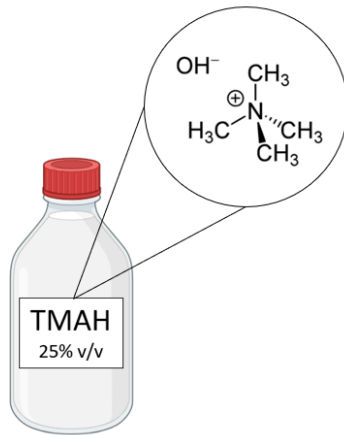


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Sample preparation: HgSeNPs & SP-ICP-MS

Reagents

- Enzymes
- Formic acid
- ...



TETRAMETHYLAMMONIUM
HYDROXIDE

Energy

- Sonication (bath/probe)
- Heating-block
- Heat-shaking
- ...



— Mvfi 18

Steps

- Defatting
- Incubation with reagents
- Application a source of energy
- Ultracentrifugation
- ...



- CO_2 , CH_4 , N_2O , HFC , PFC , SF_6 , NF_3

*Lack of NP
standards/
reference materials/
certified reference
materials*

- ~~SCHE~~ € †, , π, , † π · Š

*Lack of NP
standards/
reference materials/
certified reference
materials*

- *Complementary techniques/methods*
- *Hyphenated techniques and/or ICP-time of flight (TOF)-MS detection*
- *Stablish a common comparison framework*
- *Study of current available reference materials for classical mercury species*

Acknowledgements



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Critical assessment of sample preparation methods for the study of HgSe nanoparticles in fish tissues by SP-ICP-MS





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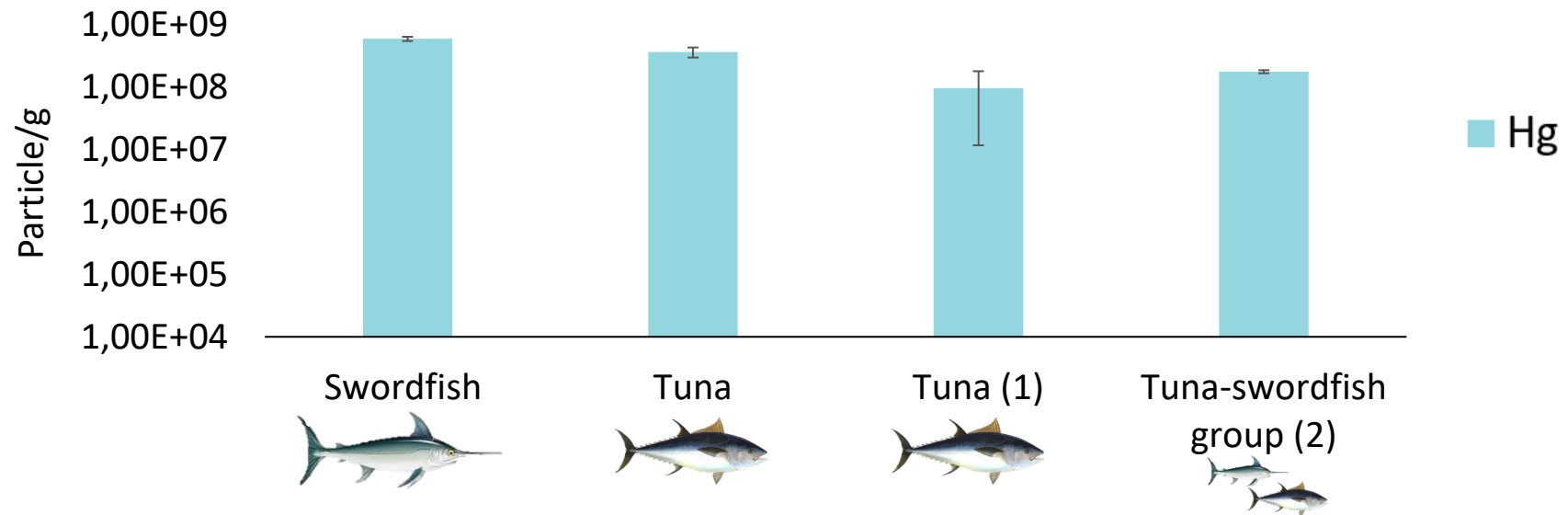
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Comparison with other NP studies in fish muscle




ICP analysis		Tissue	n	Hg (mg kg ⁻¹)	Se (mg kg ⁻¹)	Se:Hg molar ratio
Swordfish		Muscle	3	2.66 ± 0.22	3.34 ± 0.13	3.1
Tuna		Muscle	3	2.39 ± 0.11	3.38 ± 0.14	3.6
Tuna (1)		Muscle	9	2.5 ± 1.5	3.6 ± 2.9	3.7
Swordfish-tuna group (2)		Muscle	44	0.656	-	-



(1) Wiech et al., *Journal of Hazardous Materials* (2024)

(2) Suzuki et al., *Environmental pollution* (2022)

Application to real samples

ICP analysis	Tissue	n	Hg (mg kg ⁻¹)	Se (mg kg ⁻¹)	Se:Hg molar ratio
Tambaqui 	Muscle 	3	7.2 ± 1.2	6.581 ± 0.083	2.3
	Liver 	3	9.86 ± 0.57	16.8 ± 3.2	4.3

