Supplementary Material

Determination of Pb(II) Ions in Water by Fluorescence Spectroscopy Based on Silver Nanoclusters

Luca Burratti [1,*], Valentin Maranges [1], Michele Sisani [2], Eziz Naryyev [1], Fabio De Matteis [1], Roberto Francini [1] and Paolo Prosposito [1]

[1] Department of Industrial Engineering, University of Rome Tor Vergata, Via del Politecnico 1, 00133 Rome, Italy
[2] Prolabin & Tefarm S.r.l., 06134 Perugia, Italy
[*] Correspondence: luca.burratti@uniroma2.it

Error Analysis

S1. Lab Tools and Their Errors

- Balance → δ_m = 0.1 mg
- 1000 mL pipette → systematic error (δ_s) = 0.012 mL, random error (δ_r) = 0.008 mL, total error $\delta_T^{1000} = \sqrt{\delta_s^2 + \delta_r^2} = 0.014\ mL$
- 10 μL pipette → systematic error (δ_s) = 0.12 μL, random error (δ_r) = 0.08 μL, total error $\delta_T^{10} = \sqrt{\delta_s^2 + \delta_r^2} = 0.14\ \mu L$
- 10 mL graduated cylinder → δ_V^{10} = 0.1 mL
- 50 mL graduated cylinder → δ_V^{50} = 0.5 mL

S2. Preparation of *Reference* Solution

In a typical synthesis of *Reference* solution 39.7 ± 0.1 mg of Pb precursor salt were dissolved in 20.00 ± 0.14 mL

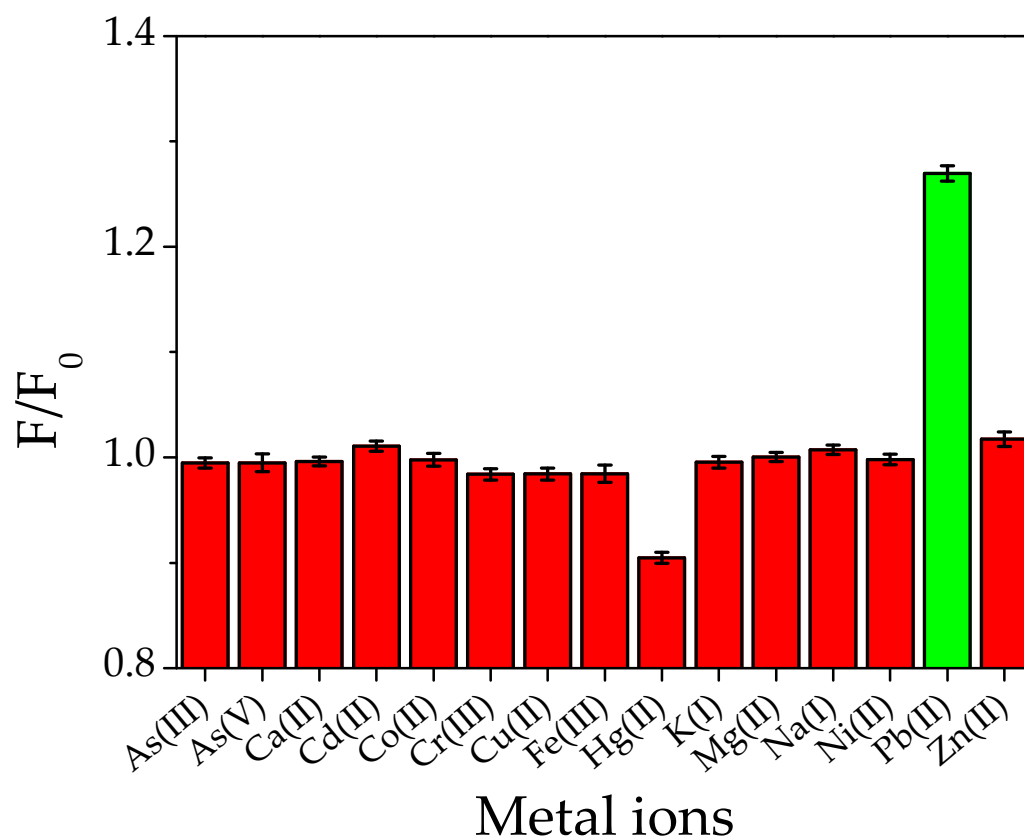Molarity and error propagation of molarity:

$$C = \frac{mass(g)}{volume\ (l) * PM\ \left(\frac{g}{mol}\right)} \rightarrow \frac{\delta_C}{C} = \sqrt{\left(\frac{\delta m}{m}\right)^2 + \left(\frac{\delta V}{V}\right)^2} \rightarrow \delta_C = C * \sqrt{\left(\frac{\delta m}{m}\right)^2 + \left(\frac{\delta V}{V}\right)^2}$$

where the PM is treated as a constant.

Reference → 6000 ± 45 μM

S3. Selectivity and Interference Tests

Figure S1 shows the selectivity tests on the AgNCs-PMAA colloidal solution, while the Figure S2 shows the interference tests. The concentration of metal ions in both experiments was set at 2.5 μM. In both graphs, the Pb(II) contribution has been underlined with green color of the bar.

Figure S1. Selectivity tests on the AgNCs-PMAA solution.
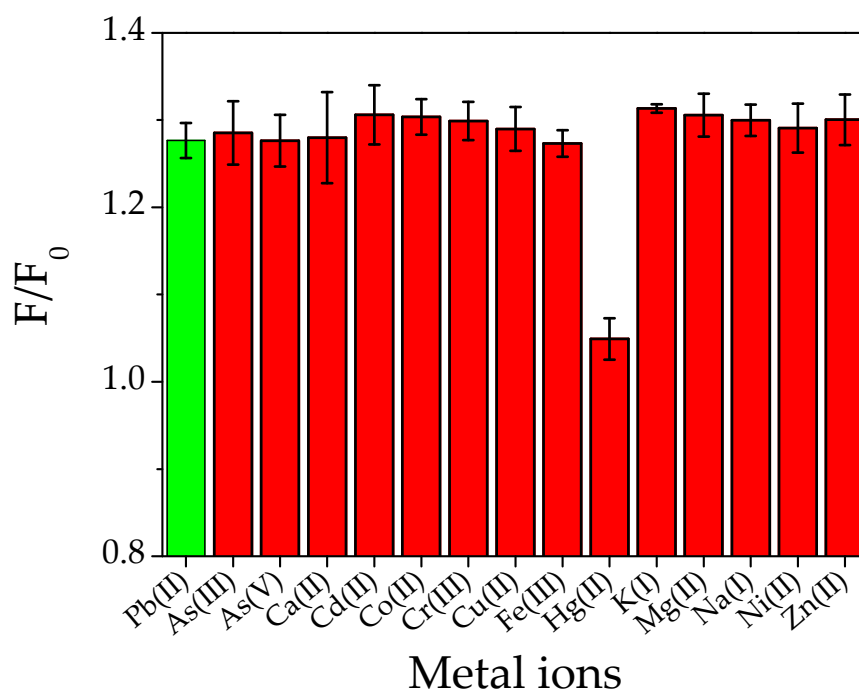
Figure S2. Interference tests on the AgNCs-PMAA solution.

S4. Preparation of *Test* Solutions

To prepare the *Test* solutions the proper masses of Pb(NO₃)₂ (PM: 331.21 g/mol) were weighted as follow:

Molarity and error propagation of molarity:

$$C = \frac{mass(g)}{volume\,(l) * PM\,(\frac{g}{mol})} \rightarrow \frac{\delta_C}{C} = \sqrt{\left(\frac{\delta m}{m}\right)^2 + \left(\frac{\delta V}{V}\right)^2} \rightarrow \delta_C = C * \sqrt{\left(\frac{\delta m}{m}\right)^2 + \left(\frac{\delta V}{V}\right)^2},$$

the PM is treated as a constant.

- A → 22.7 ± 0.1 mg in 5.00 ± 0.03 mL of deionized water → 13.7 ± 0.1 mM
- B → 14.6 ± 0.1 mg in 5.33 ± 0.03 mL of deionized water → 8.27 ± 0.08 mM
- C→ 6.4 ± 0.1 mg in 5.16 ± 0.03 mL of deionized water → 3.72 ± 0.06 mM

Solution D was diluted from solution C → 0.154 mL of solution C and 0.846 mL of deionized water, using 1000 mL pipette. For the dilution, we used the following formula

$$C_i * V_i = C_f * V_f \tag{S1}$$

where V_i e V_f are the initial volume and the final volume, respectively, and the C_i and C_f are the initial and final concentration, respectively in mM. Thus $Cf = Ci * Vi/Vf$ and the error propagation will be:

$$\frac{\delta_{Cf}}{Cf} = \sqrt{\left(\frac{\delta Vi}{Vi}\right)^2 + \left(\frac{\delta Ci}{Ci}\right)^2 + \left(\frac{\delta Vf}{Vf}\right)^2} \rightarrow \delta_{Cf} = C_f * \sqrt{\left(\frac{\delta Vi}{Vi}\right)^2 + \left(\frac{\delta Ci}{Ci}\right)^2 + \left(\frac{\delta Vf}{Vf}\right)^2} \tag{S2}$$

- D → 0.57 ± 0.05 mM

S5. Calibration Curve

The calibration points are listed in the Table 1 of the manuscript, to add the desired volume in the cuvette, 10 μL pipette has been used practicing one injection for the concentrations up to 10 μL and two for 30 μM and 40 μM. To estimate the concentration in the cuvette the formula (S1) has been applied and the same error propagation has been used.

The logarithm fit has been performed using the software *Origin Pro 8.5*, a logarithm function $y = a + b*ln(x)$ was used. In our case, y represents the ratio F/F_0 and x the Pb(II) concentration.

In the case of F_0, it is possible to distinguish two sources of error: the first belongs to the distribution of the measurements [$\delta_{dis.}$] and the second is the slow degradation of the colloidal solution due to the environmental temperature [$\delta_{sol.}$]. To determine $\delta_{dis.}$, the formula S3 was applied:

$$\delta_{dis.} = \frac{\sigma}{\sqrt{N}} * k_{(\nu,N)} \tag{S3}$$

where, σ is the standard deviation, N is the number of measurements, and k is the factor from the Student's law. The value of k factor mainly depends of number of measurements and the desired level of confidence (ν). In our case, the number of measurements were N = 3, and ν = 68% was selected, thus k = 1.3862.

The semi-dispersion represents the error from solution:

$$\delta_{sol.} = \frac{F_0^{start} - F_0^{end}}{2} \tag{S4}$$

where F_0^{start} represents the average value of the maximum intensity of the 3 spectra taken at the beginning of the measure session, while F_0^{end} is that at the end of the session. Combining these two error sources, we obtain the total error for F_0:

$$\delta_{F_0} = \sqrt{\delta_{dis.}^2 + \delta_{sol.}^2} \tag{S5}$$

Also in the case of samples contaminated by Pb(II), the same error sources are present, but on the contrary, the solution increases the emission as time progresses.

An approximation was necessary. As shown in the Figure 3 of the manuscript the rate of interaction depends on the Pb(II) concentration, to short and simplify the experimental procedure, we have applied the relative error calculated for the 30 μM point

to the remaining concentrations. The same formulas (S3 and S4) was applied to determine the error for F, obtaining:

$$\delta_F = \sqrt{\delta_{dis.}^2 + \delta_{sol.}^2} \tag{S6}$$

Finally, to estimate the error of the ration F/F0 it is a simple propagation of a ratio:

$$\frac{\delta_{F/F_0}}{F/F_0} = \sqrt{\left(\frac{\delta F_0}{F_0}\right)^2 + \left(\frac{\delta F}{F}\right)^2} \tag{S7}$$

Regarding the error of Pb(II) inside the cuvette, this can be obtained using the formula *(S2)* where $C_i \pm \delta C_i$ is the *Reference* concentration, $V_i \pm \delta V_i$ represents the initial volume and $V_f \pm \delta V_f$ is the final volume in the cuvette.

S6. Linearization of Logarithm Function

The error of $\ln\{[Pb(II)]\}$ was determined applying the general propagation of a function $q = q(x, \dots, z)$ of many variables *(x, …, z)* [33]:

$$\delta q = \sqrt{\left(\frac{\partial q}{\partial x}\delta x\right)^2 + \cdots + \left(\frac{\partial q}{\partial z}\delta z\right)^2} \tag{S8}$$

Thus:

$$\delta \ln\{[Pb(II)]\} = \sqrt{\left(\frac{\partial \ln\{[Pb(II)]\}}{\partial [Pb(II)]} * \delta [Pb(II)]\right)^2} \tag{S9}$$

The **Error! Reference source not found.** shows the calibration points in [Pb(II)] and $\ln\{[Pb(II)]\}$ and their errors.

Table S1. Pb(II) concentrations and their errors, in normal and logarithm forms.

[Pb(II)] in μM	δ[Pb(II)] in μM	ln{[Pb(II)]} in ln(μM)	δln{[Pb(II)]} in ln(μM)
2.5	0.3	0.92	0.12
5.0	0.3	1.61	0.06
10.0	0.4	2.30	0.03
20.0	0.4	3.00	0.02
30.0	0.5	3.40	0.02
40.0	0.6	3.96	0.01

The linearization is accomplished by the following formula:

$$\frac{F}{F_0} = a + b \ln \{[Pb(II)]\} \tag{S10}$$

where *a* and *b* are the intercept and the slope of the fitted curve, respectively. The **Error! Reference source not found.** shows the obtained parameters.

Table S2. Parameters calculated by the fitting process.

Fit Number	a	b	R²
1	1.11 ± 0.05	0.33 ± 0.02	0.984
2	1.07 ± 0.05	0.35 ± 0.02	0.989
3	1.10 ± 0.01	0.412 ± 0.005	0.999

S7. Quantum Yield

To determine the quantum yield (QY) of AgNCs-PMAA with and without Pb(II), we used the following formula:

$$\eta_s = \frac{\Phi_s}{\Phi_R} \cdot \frac{A_R}{A_s} \cdot \frac{n_s^2}{n_R^2} \cdot \eta_R \tag{S11}$$

where η represents the quantum yield, Φ is the integral of the emission curve, A indicates the absorbance value. Finally, n represents the refractive index of the solvent [in our case: water (n = 1.333) for NCs and ethanol (n = 1.361) for the reference]. Where subscripts "s" and "R" are referred to the sample and to the reference, respectively. The Table S3 shows the obtained values by exciting with λ = 430 nm, and using Rhodamine B as standard (QY = 97%).

Table S3. Parameters for the determination of quantum yield of the AgNCs samples.

Sample	Φ	A	η
Rhodamine B	5.20483	0.229794	97% [1]
AgNCs-PMAA	0.01312	0.293576	0.2%
AgNCs-PMAA + 2.5µM of Pb(II)	0.01862	0.291867	0.3%
AgNCs-PMAA + 30µM of Pb(II)	0.03108	0.292563	0.4%

[1] Literature value [31].

S8. Test Concentrations and Their Determination

To determine the concentration of *Test* solution in the cuvettes, the emission from samples was recorded, then through the linear function it was possible to determinate the [Pb(II)] in the cuvette.

To determine the error of the Pb(II) concentrations in the cuvettes, the general propagation has been applied (formula S8) to the following function:

$$[Pb(II)] = e^{(\frac{F}{F_0}-a)/b} \tag{S12}$$

Obtaining:

$$\delta[Pb(II)] = \sqrt{\left(\frac{\partial [Pb(II)]}{\partial \frac{F}{F_0}} * \delta(\frac{F}{F_0})\right)^2 + \left(\frac{\partial [Pb(II)]}{\partial a} * \delta(a)\right)^2 + \left(\frac{\partial [Pb(II)]}{\partial b} * \delta(b)\right)^2} =$$

$$= \sqrt{\left(\frac{\partial \left[e^{(\frac{F}{F_0}-a)/b}\right]}{\partial \frac{F}{F_0}} * \delta(\frac{F}{F_0})\right)^2 + \left(\frac{\partial \left[e^{(\frac{F}{F_0}-a)/b}\right]}{\partial a} * \delta(a)\right)^2 + \left(\frac{\partial \left[e^{(\frac{F}{F_0}-a)/b}\right]}{\partial b} * \delta(b)\right)^2} \tag{S13}$$

$$\sqrt{\left(\frac{e^{(\frac{F}{F_0}-a)/b}}{b} * \delta(\frac{F}{F_0})\right)^2 + \left(-\frac{e^{(\frac{F}{F_0}-a)/b}}{b} * \delta(a)\right)^2 + \left(\frac{\left(a-\frac{F}{F_0}\right) * \left[e^{(\frac{F}{F_0}-a)/b}\right]}{(b)^2} * \delta(b)\right)^2}$$

Once determined the concentration in the cuvettes, it was possible to calculate the concentration of mother solutions of *Tests* and their errors, applying the formula (S1) and (S2). Thus, V_i is the volume injected in the cuvette (16.67 µL), V_f is the total volume in the cuvette (3.01667 mL), C_f is the Pb(II) concentration determined by measuring the emission and applying the method with the linear fit. The C_i is the concentration to be determined:

$$C_i = C_f * V_f / V_i \tag{S14}$$

$$\frac{\delta_{C_i}}{C_i} = \sqrt{\left(\frac{\delta C_f}{C_f}\right)^2 + \left(\frac{\delta V_f}{V_f}\right)^2 + \left(\frac{\delta V_i}{V_i}\right)^2} \rightarrow \delta_{C_i} = C_i * \sqrt{\left(\frac{\delta C_f}{C_f}\right)^2 + \left(\frac{\delta V_f}{V_f}\right)^2 + \left(\frac{\delta V_i}{V_i}\right)^2} \quad (S15)$$

The **Error! Reference source not found.**3 shows the comparison of the *Test* concentrations determined by synthesis (considering the mass and the volume employed in the preparation of the solutions), fluorescence method and ICP-OES.
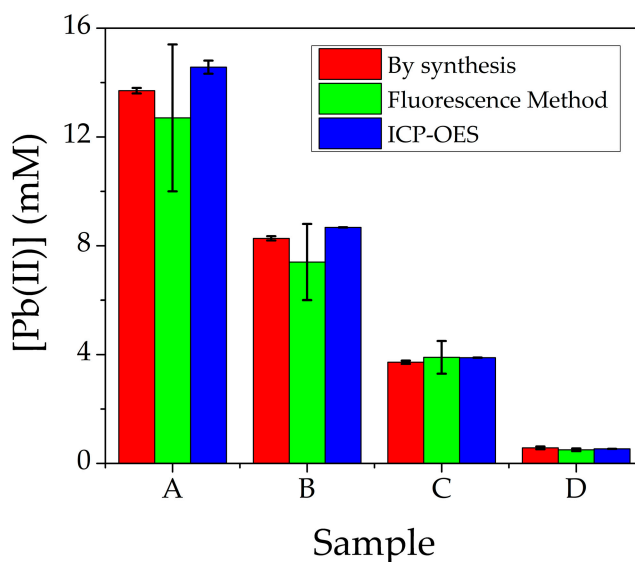
Figure S3. Concentrations of the Tests determined by synthesis (red bars), fluorescence method (green bars) and ICP-OES (blue bars).

S9. Mineral Water Tests

Two different mineral waters (indicated as W1 and W2) were polluted with the same concentration of the Test solutions (13.7, 8.27, 3.72, 0.57 mM). Our fluorescence method was applied to verify the sensitivity and selectivity of the system. The Table S4 shows the composition of mineral waters used in the experiment (appearing on the label of the bottles).

Table S4. Composition of commercial mineral waters.

Sample	Element	Concentration (mg/L)
	Nitrites	<0.002
	Na^+	42
	Ca^{2+}	100
W1	F^-	1.10
	Bicarbonates	471
	Mg^{2+}	24
	K^+	63
	Nitrates	3
	Na^+	4.9
	Ca^{2+}	87
	Cl^-	7.7
W2	F^-	<0.2
	Bicarbonates	321
	Mg^{2+}	17.6
	K^+	1.2

S10. Comparison with the Literature

Table S5. Comparative table about the precision (RSD) of different fluorescent materials.

System	RSD Range	Metal Ion	Ref.
AuNCs-GSH	8.1–12.7%	Pb(II)	[41]
"	6.8–12.8%	Hg(II)	"
AgNCs-Tryptophan	1.73–4.72%	Cu(II)	[42]
Cu/AgNCs-DNA	8–10%	Hg(II)	[43]
AuNCs + CQDs	2.8–3.1%	Pb(II)	[44]
"	2.9–3.3%	Cu(II)	"
AuNCs-Trypsin	0.39–45%	Co(II)	[45]
"	0.32–67%	Cu(II)	"
"	0.16–49%	Hg(II)	"
AuNCs + CQDs	1.02–2.80%	Hg(II)	[46]
AgNCs-PMAA	10–24%	Pb(II)	This work