Communication

Fluorescence and naked-eye detection of Pb²⁺ in drinking water using a low-cost ionophore based sensing scheme

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Abstract: Drinking water contamination of lead from various environmental sources, leaching consumer products and intrinsic water-pipe infrastructure is still today a matter of great concern. Therefore, new highly sensitive and convenient Pb^{2+} measurement schemes are necessary, especially for in-situ measurements at a low-cost. Within this work dye/ionophore/Pb^{2+} co-extraction and effective water phase de-colorization was utilized for highly sensitive lead measurements and sub-ppb naked-eye detection. A low-cost ionophore Benzo-18-Crown-6-ether was used, and a simple test-tube mix and separate procedure was developed. Instrumental detection limits were in the low ppt region (LOD=3, LOQ=10), and naked-eye detection was 500 ppt. Note, however, that this sensing scheme still has improvement potential as concentrations of fluorophore and ionophore were not optimized. Artificial tap-water samples, leached by a standardized method, demonstrated drinking water application. Implications for this method are convenient in-situ lead ion measurements.

Keywords: lead ions, fluorescence detection, ionophore, Benzo-18-Crown-6-ether, drinking water

1. Introduction

Environmental lead contamination in water, food and consumer products are still alarming problems [1–5][6][7]. Therefore, new highly sensitive and selective lead measurements are necessary, in particular for convenient field measurements at a low-cost [1–5][8]. Currently common and reliable detection methods for lead ions in drinking water and many other products mostly depend on laboratory analysis with instruments such as ICP-OES or ICP-MS or graphite furnace atomic absorption spectroscopy, which are by no means simple, cheap or portable [2][9]. A recurrent reason for lead contaminated drinking water within cities is ageing water supply infrastructure such as in the Flint water crisis [10], which also revealed dangers regarding lead exposure, especially for children [10]. The US Environmental Protection Agency (EPA) says that 10 – 20 % of US residents' exposure to lead comes from contaminated water and babies can even get up to 60% of their exposure to lead by drinking formula mixed with contaminated water. EPAs permissible limit of lead in drinking water is 15 ppb [11], while The World Health Organization (WHO) recommendation is even lower at 10 ppb [12].

Within this work dye/ionophore/Pb^{2+} co-extraction and effective water phase de-colorization, and consequent fluorescence decline, is utilized for highly sensitive lead measurements and sub-ppb naked-eye detection. A simple test-tube mix, shake and separate procedure was developed. Prize per sample is extremely competitive and without any cost-optimization the total cost for the chemicals for one sample is ~0.1 US$. Miniaturized instrumentation for high-performance field measurements can be built for less than 50 UD$. Generality and easy ionophore replacement is not

claimed here and the opposite is actually demonstrated with another more common (and 100-times more expensive) lead ionophore. Though, carefully considered (of the whole system) most ionophores should be possible utilize in a similar way. A real life sample, a "lead-free" manometer for a coffee machine, was tested according to a standardized leaching test protocol and cross-correlated with ICP-OES with good results. Lead contamination is especially challenging in for example coffee machines, due to the long-term heating of water and consequent accelerated leaching of lead and other species.

2. Materials and Methods

2.1 Instruments

The fluorescence experiments were performed on a FluoroMax 4 spectrofluorometer from Jobin Yvon (Horiba group). Excitation and emission matrix spectra were collected with slit widths corresponding to 2 nm bandpass; integration times were set to 0.1 s and 10 nm steps were used.

2.2 Chemicals

1-Octanol, Merocyanine 540 (90 %) and Benzo-18-Crown-6-ether (98 %) were all reagent grade and purchased from Sigma-Aldrich. MilliQ water (electric resistivity > 18MΩcm−1) was obtained from a Millipore water purification system. For standards 1000 mg/L Pb, ($Pb(NO_3)_2$ in H_2O) Titrisol® (109969) was used and diluted with MillQ water.

2.3 Experimental

2 ml octanol (with Benzo-18-Crown-6-ether 0.5 mg/ml), 1 ml Merocyanine 540 (15 μM in MilliQ water) and 1 ml water sample is mixed thoroughly in a 5 ml glass test-tube, which gave a pink opaque solution (emulsion), that was allowed to separate for 15 minutes. After which naked-eye and/or fluorescence measurements were performed directly in the test-tube lower water phase.

3. Results

The research conducted here was initiated with attempted developments of a previously published nanoparticle enhanced ammonium and ammonia optical sensor [13–15]. The intention was to expand the portfolio for ionophore/coextraction based optical chemosensors with lead sensors. Several lead ionophores were tested without success, both with and without plasmonic nanoparticles. Therefore, the principles used and developed here were initially performed to understand the sensing mechanism at a macroscopic level in a test tube.

Figure 1 shows test-tubes with separated solutions with zero and 500 ppm lead ions. Also, the proposed sensing mechanism is illustrated in Fig. 1, where the Benzo-18-Crown-6-ether collects Pb^{2+} ions and ion pairs with two Merocyanine 540 (one negative charge each) molecules and co-extracts into the less polar octanol phase, with consequent de-colorization and fluorescence decrease in the water phase. Resulting excitation/emission fluorescence matrices are shown in Fig. 2 A-D for 0/10/500 ppt and 500 ppm lead ions, and a declining fluorescence trend is realized. Another Pb^{2+} ionophore (Lead IV, Selectophore) was tested in the same way with much lower responses. Naked eye detection of 500 ppt lead ions was assessed with both ionophores and pictures are shown in Fig. 3 A, with an advantage for Benzo-18-Crown-6-ether. This is an indication that all ionophores will not perform on this high level by default in this set-up.
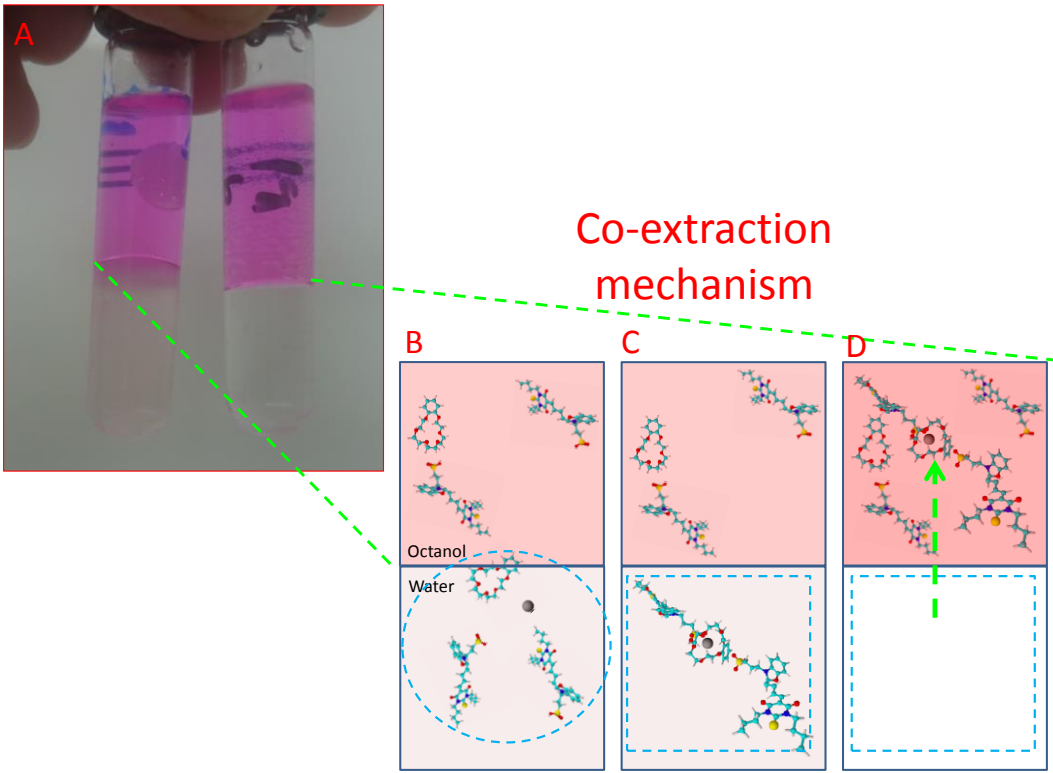
89
90   **Figure 1**   A) test tubes with separated octanol/water sensor cocktails: zero level (left) and 500 ppm Pb^{2+}. B)
91   cartoon showing the co-extraction three step principle by B) Pb^{2+} introduction near one
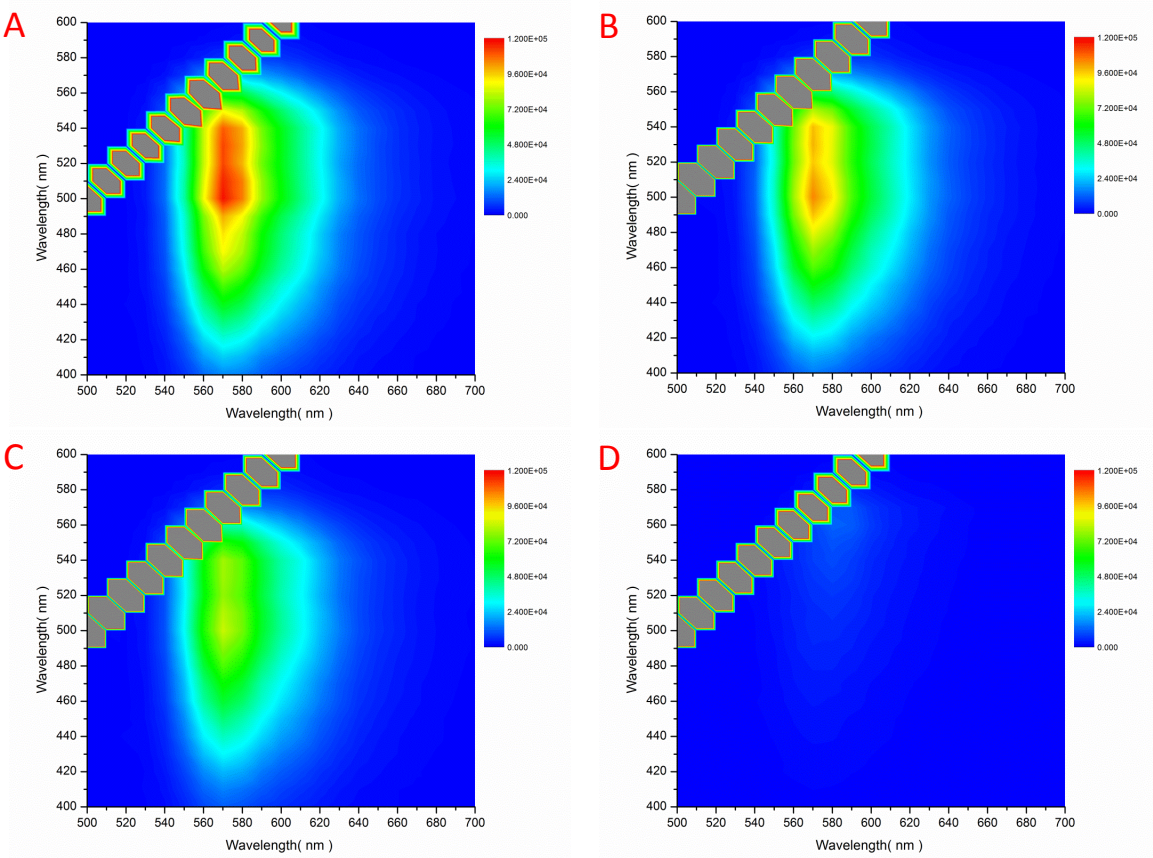92   Benzo-18-Crown-6-ether and two Merocyanine 540 molecules. C) Complexation D) Phase transfer.

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94   **Figure 2**   Fluorescence excitation/emission matrix scans of the water phase for Pb^{2+} ion concentrations of: A) 0 B)
95   10 ppt C) 500 ppt D) 500 ppm. Note that the intensity scales are set the same.
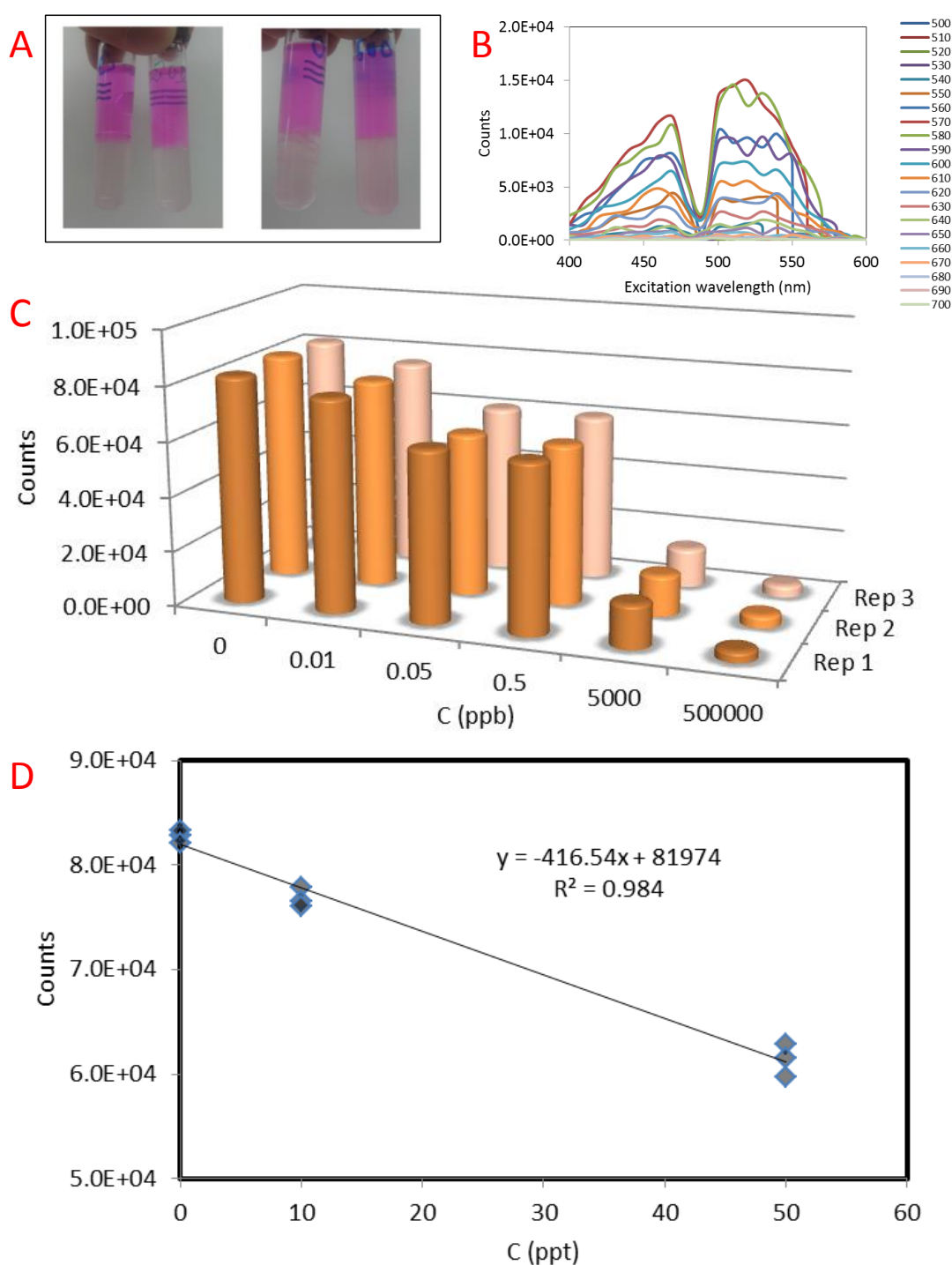
Figure 3 A) Naked eye detection of 500 ppt Pb^{2+} (left tube), and with less sensitive Lead IV ionophore (right) as comparison. B) Finding optimized fluorescence wavelengths. C) Repeatability at different concentrations, first repetition on each concentration in dark orange, second in medium orange and third in light orange. D) Low ppt level calibration curve for the optimized Ex480/Em580 wavelengths. LOD=3 ppt LOQ=10 ppt.

To find maximum signal responses difference excitation spectra for Zero – 10 ppt was calculated (Fig. 3B). Ex520/Em570 nm showed maximum response. However, often this may not be the same as best signal to noise ratio, which can be found by dividing the signal by zero level standard deviation [16][17]. Here, optimum S/N ratio → S/N= 9.8 was found for 10 ppt response at Ex480/Em580 nm, which also demonstrated a high signal in Fig. 3B. The repeatability of this wavelength pair at different concentrations are shown in Fig. 3C. To calculate limits of detection

109   (LOD) and quantification (LOQ) a calibration curve for the low ppt range was constructed (Fig. 3D).
110   The response at 0 – 50 ppt was linear with a good correlation coefficient (R^2 = 0.984) and excellent
111   detection limits were calculated (LOD=3 ppt and LOQ=10 ppt). A real sample: a coffee machine
112   manometer was leached according to the requirements in SS-EN 16889:2016. The quantity of
113   migrated lead in the test water shall not exceed the guideline value of 0.01 mg/kg (10 ppb). The
114   leached manometer ICP-OES result was 3.5 ±0.7 ppb, while the fluorescence method result was 3.0
115   ±0.04 ppb, i.e. in good correlation.
116   A (or the) major interference Cu^{2+} was experimentally tested and compared with Pb^{2+} with
117   regards to fluorescence response with the Benzo-18-Crown-6-ether as ionophore. The result of this is
118   shown in figure 4.
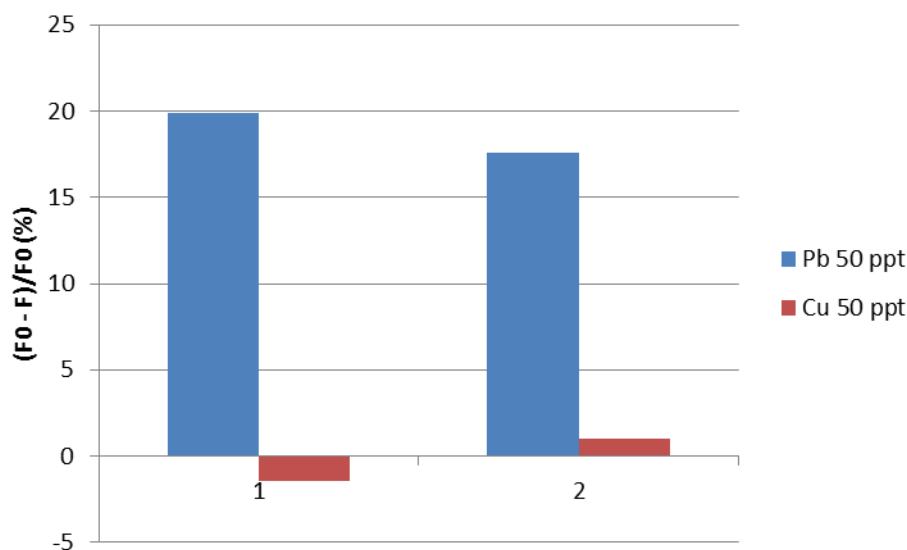119

120
121   **Figure 4**   Relative fluorescence signal response for duplicate samples of 50 ppt Pb^{2+} (blue) and Cu^{2+} (red),
122   respectively. Benzo-18-Crown-6-ether was used as ionophore for both ions.

123   **4. Discussion**

124   With regards to selectivity the Benzo-18-Crown-6-ether has been demonstrated as highly
125   selective over its five major interferences and demonstrated an average selectivity coefficient for
126   those of pK_{5A} = 5.34 (Ion:pK; Ca^{2+}:5.2; Mg^{2+}:6.3; Cu^{2+}:4.7; Zn^{2+}: 4.9; Cd^{2+}:5.6), which was almost as good
127   as the more commonly used Lead IV ionophore (Selectophore™) [18][19]. This was also the main
128   reason to involve Benzo-18-Crown-6-ether within this study. The detection method is different, but
129   the complexation and co-extraction take place similarly, hence, similar selectivity is expected. A brief
130   selectivity test towards sodium ions was conducted and demonstrated that 1 ppm Na^+ gave the same
131   response as 100 ppt Pb^{2+} ions indicating 10000 or 5 orders of magnitude in signal response increase
132   (i.e. similar to the pK_{5A} = 5.34 mentioned above) [19]. For the major interference Cu^{+2} low level
133   detection (50 ppt) was compared (Fig. 4), with 18 – 20 % relative signal response for Pb^{2+} and -1 – 1 %
134   for Cu^{2+}.
135   Comparisons and a brief overview of literature shows that laboratory instrumental techniques
136   for lead measurements commonly involve ICP-MS (LOD low ppt range) and ICP-OES (LOD low
137   ppb range) [20]. Among potential ultra-sensitive in-situ sensing/detection techniques for drinking
138   water one of the better competitors demonstrate a naked-eye detection of 2 ppb and an instrumental
139   semi-quantitative detection of 190 ppt (0.19 ng/ml) [9]. Further, a recent article demonstrated an
140   interesting lab in syringe concept for in-situ detection of lead ions with a LOD = 23 nmol L^{-1} (5 ppb)
141   [5].   Another technique, a label-free impedimetric sensing system based on DNAzyme and ordered
142   mesoporous carbon–gold nanoparticles, showed a LOD of 0.2 nmol L^{-1} (40 ppt) [21]. Another
143   DNAzyme based detection scheme demonstrated a detection limit of 0.7 nM (130 ppt) [22]. One
144   among the top-notch fluorescence techniques for Pb^{2+} ions demonstrated a detection limit of 1

145 nmol L⁻¹ (200 ppt), using a polyguanine (G(33))/terbium ions (Tb(3+)) conjugate [23]. The best
146 fluorescence detection we found to date are another DNAzyme technique showing a detection limit
147 of 0.06 nmol L⁻¹ (12 ppt) [24]. Additionally, there are convenient commercial colorimetric test sticks
148 for Pb²⁺ detection, for example MQuant™ from Merck. However, these test sticks are restricted by a
149 detection limit on the order of low ppm levels.

5. Conclusions

151 Ultra-sensitive fluorescence and naked-eye detection of Pb²⁺ in drinking water was
152 demonstrated using low-cost Benzo-18-Crown-6-ether as selection ionophore. A simple test-tube
153 mix and separate technique was successfully developed. Instrumental detection limits were in the
154 low ppt region (LOD=3, LOQ=10), and naked-eye detection was on the order of 500 ppt. Coffee
155 machine water samples, leached by a standardized method, correlated well with ICP-OES.
156 Implications are simple and ultra-sensitive lead ion measurements in the field.

158 **Conflicts of Interest:** The authors declare no conflict of interest.
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