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Communication

Fluorescence and naked-eye detection of Pb^{2+} 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. Low-cost ionophore Benzo-18-Crown-6-ether was used, and a simple test-tube mix, shake 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, content and control 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 can be 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 turn-off, is utilized for highly sensitive lead measurements and sub-ppb naked-eye detection. A simple test-tube mix, shake and separate procedure were developed. Sample prizes are 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

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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. The low limits of lead are especially challenging in for example coffee machines and such, due to long-term heating of water and consequent 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 our 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 gets co-extracted into the octanol phase, with consequent de-colorization and fluorescence decrease in the water phase. Resulting excitation/emission fluorescence matrixes are shown in Fig. 2 A-D for 0/10/500 ppt and 500 ppm lead ions, and a decreasing fluorescence trend is revealed even for the 10 ppt sample. 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, again with a clear 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.

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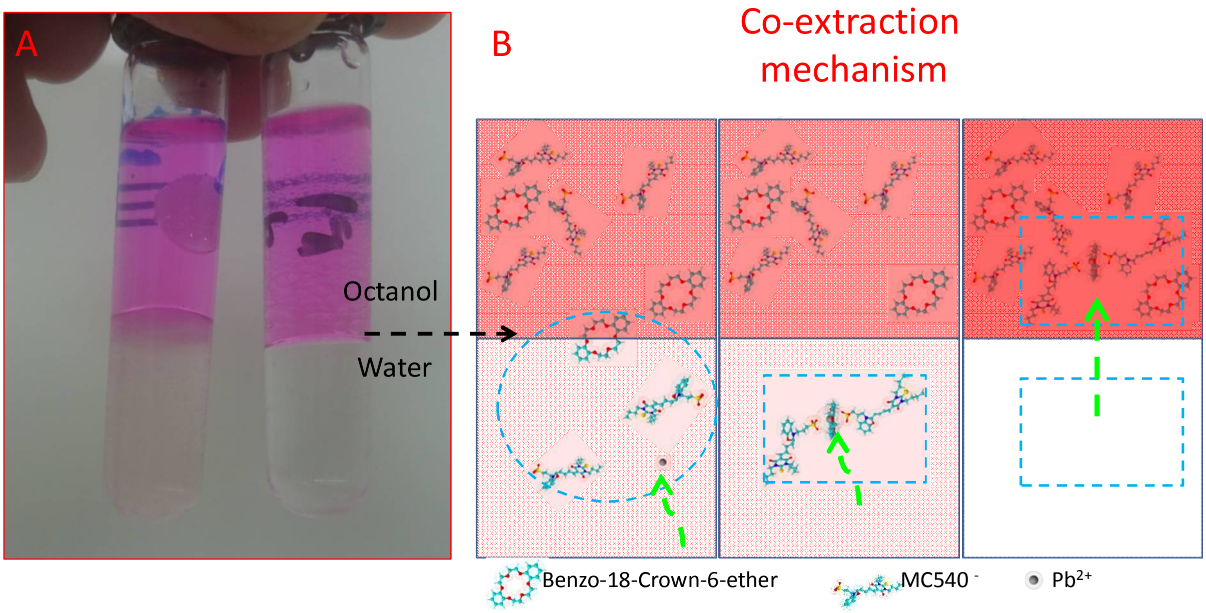

Figure 1 A) test tubes with separated octanol/water sensor cocktail solutions: zero level (left tube) and 500 ppm Pb^{2+} (diluted reference standard solution). B) cartoon showing the co-extraction three step principle by Pb^{2+} introduction, complexation and phase transfer. Green arrows indicate lead ion and the formed complex transferences.

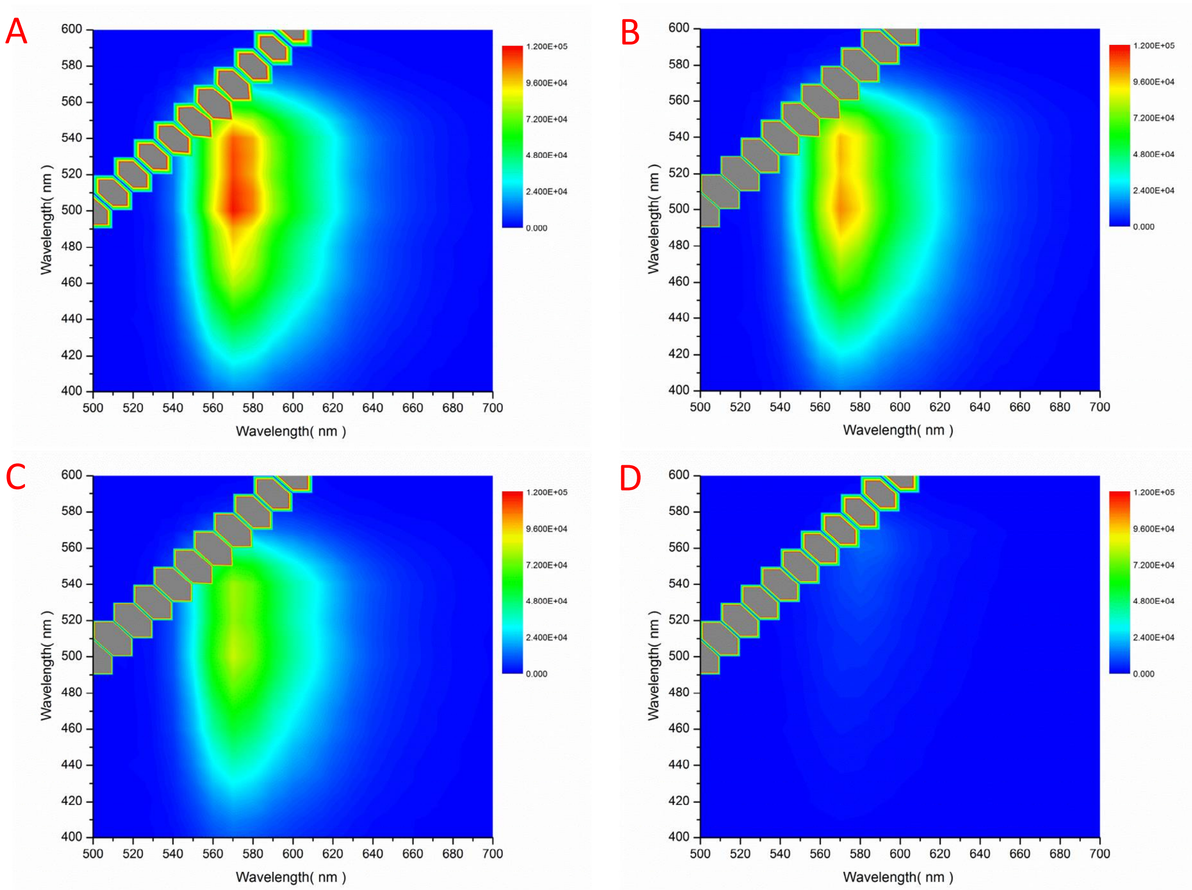

Figure 2 Fluorescence excitation/emission matrix scans of the water phase for Pb^{2+} ion concentrations of: A) 0 B) 10 ppt C) 500 ppt D) 500 ppm. Note that the intensity scales are set the same.

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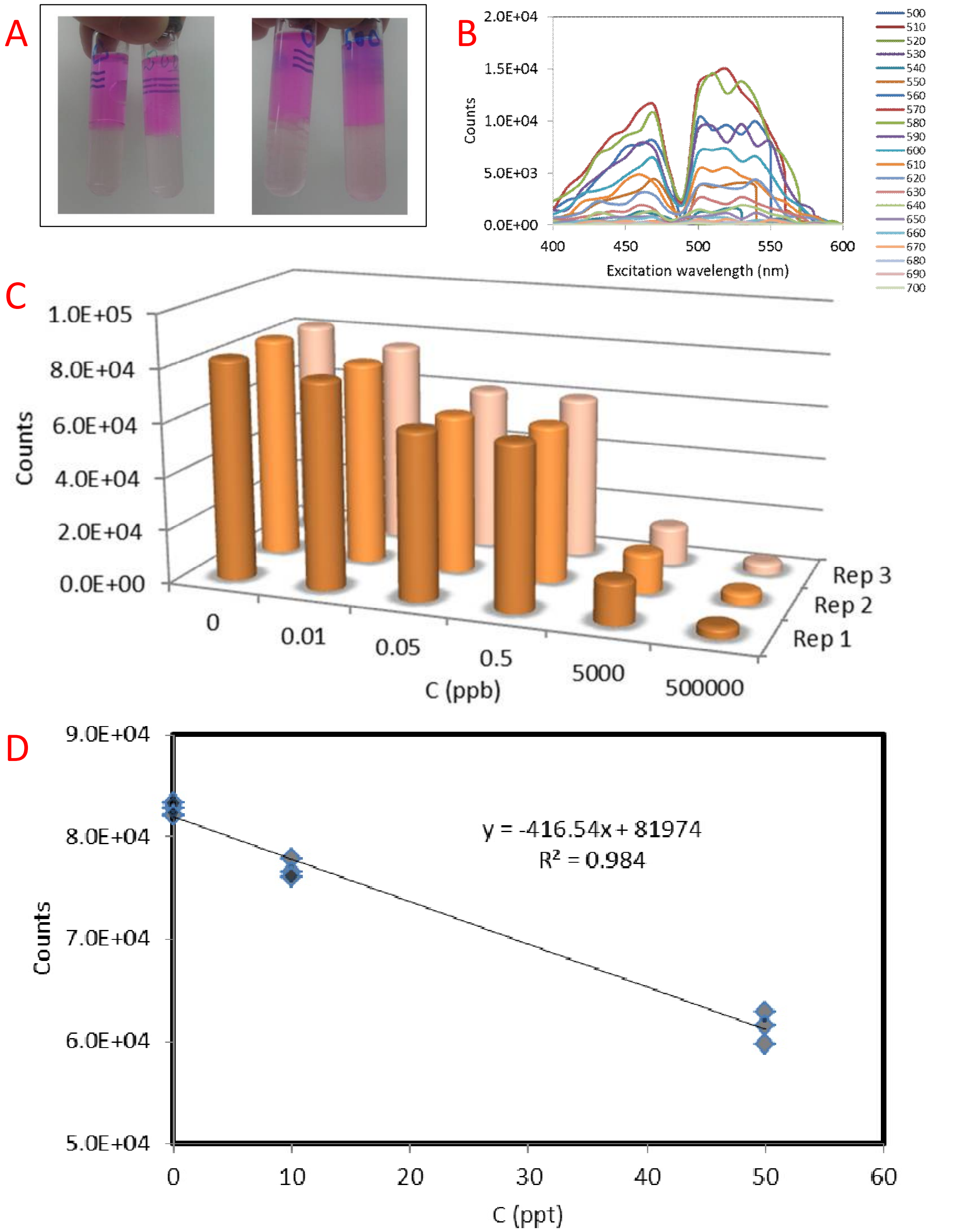

Figure 3 A) Naked eye detection of 500 ppt Pb^{2+} (left tube), and with less sensitive Lead IV ionophore (right) as comparison. B) Finding optimized fluorescence wavelengths. C) Repeatability at different concentrations and D) Low ppt level calibration curve for the optimized Ex480/Em580 wavelengths. 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 searching for highest signal divided 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

(LOD) and quantification (LOQ) a calibration curve for the low ppt range was constructed (Fig. 3D). The response at 0 – 50 ppt was linear with a good correlation coefficient (R^2 = 0.984) and excellent detection limits (LOD=3 ppt and LOQ=10 ppt). A real sample: a coffee machine manometer was leached according to the requirements in SS-EN 16889:2016. The quantity of migrated lead in the test water shall not exceed the guideline value of 0.01 mg/kg (10 ppb). The leached manometer ICP-OES result was 3.5 ±0.7 ppb, while the fluorescence method result was 3.0 ±0.04 ppb, i.e. in relatively good correlation.

4. Discussion

With regards to selectivity the Benzo-18-Crown-6-ether has been demonstrated as highly selective over its five major interferences and demonstrated an average selectivity coefficient for those of pK_{5A} = 5.34, which was almost as good as the more commonly used Lead IV inonophore (Selectophore™) [18][19]. This was also the main reason to involve Benzo-18-Crown-6-ether within this study. The detection method is different, but the complexation and co-extraction take place similarly, hence, similar selectivity is expected. A brief selectivity test towards sodium ions was conducted and demonstrated that 1 ppm Na^+ gave the same response as 100 ppt Pb^{2+} ions indicating 10000 or 5 orders of magnitude in signal response increase (i.e. similar to the pK_{5A} = 5.34 mentioned above) [19].

In comparisons with literature and for a brief overview, laboratory instrumental techniques for lead measurements commonly involve ICP-MS (LOD low ppt range) and ICP-OES (LOD low ppb range) [20]. Among potential ultra-sensitive in-situ sensing/detection techniques for drinking water one of the better competitors demonstrate a naked-eye detection of 2 ppb and an instrumental semi-quantitative detection of 190 ppt (0.19 ng/ml) [9]. Further, a recent article demonstrated an interesting lab in syringe concept for in-situ detection of lead ions with a LOD = 23 nmol L^{-1} (5 ppb) [5]. Another technique, a label-free impedimetric sensing system based on DNAzyme and ordered mesoporous carbon–gold nanoparticles, showed a LOD of 0.2 nmol L^{-1} (40 ppt) [21]. Another DNAzyme based detection scheme demonstrated a detection limit of 0.7 nM (130 ppt) [22]. One among the top-notch fluorescence techniques for Pb^{2+} ions demonstrated a detection limit of 1 nmol L^{-1} (200 ppt), using a polyguanine (G(33))/terbium ions (Tb(3+)) conjugate [23]. The best fluorescence detection we found to date are another DNAzyme technique showing a detection limit of 0.06 nmol L^{-1} (12 ppt) [24].

5. Conclusions

In conclusion, practical and ultra-sensitive fluorescence and naked-eye detection of Pb^{2+} in drinking water was demonstrated using low-cost Benzo-18-Crown-6-ether as ionophore for principles of co-extraction. A simple test-tube mix and separate technique and was successfully developed. Instrumental detection limits were in the low ppt region (LOD=3, LOQ=10), and naked-eye detection was 500 ppt or better. Coffee machine water samples, leached by a standardized method, correlated well with ICP-OES. Implications are simple and ultra-sensitive lead ion measurements in the field.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: title, Table S1: title, Video S1: title.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "conceptualization, X.X. and Y.Y.; methodology, X.X.; software, X.X.; validation, X.X., Y.Y. and Z.Z.; formal analysis, X.X.; investigation, X.X.; resources, X.X.; data curation, X.X.; writing—original draft preparation, X.X.; writing—review and editing, X.X.; visualization, X.X.; supervision, X.X.; project administration, X.X.; funding acquisition, Y.Y.", please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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