



Visual detection of Al³⁺ ions using conjugated copolymer-ATP supramolecular complex



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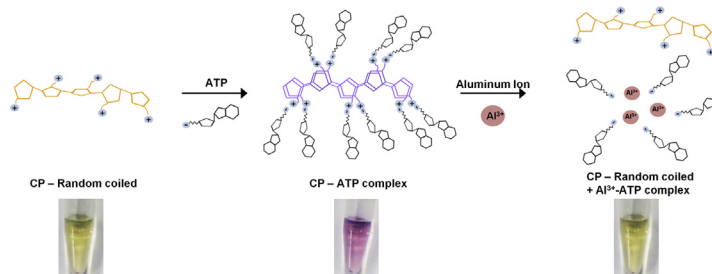
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HIGHLIGHTS

- A novel water soluble polythiophene copolymer that demonstrates colorimetric response for Al³⁺ is proposed.
- Optimized ratio of two polythiophene monomers is utilized for selective detection of Al³⁺.
- Naked eye detection of Al³⁺ is demonstrated without involving sophisticated instrumentation.
- Lowest concentration of Al³⁺ for naked eye detection is ~4 μM, which is below the threshold levels.

GRAPHICAL ABSTRACT



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ABSTRACT

A colorimetric Al³⁺ sensor based on fluorescence recovery of a conjugated copolymer-ATP complex is proposed. An optimized ratio of two polythiophene (PT) monomers is utilized to synthesize copolymer (CP) that yielded maximized colorimetric response for Al³⁺ in deionized (DI) and tap water. The electrostatic disassembly of CP-ATP upon addition of Al³⁺ led to an evident visual color change. The lowest concentration of Al³⁺ for naked eye observation is around 4 μM, which is below the threshold levels in drinking water according to European Economic Community (EEC) standard. Besides, the proposed assay showed a similar response to Al³⁺ in tap water. The proposed methodology showed selective and sensitive detection for Al³⁺ in analytically relevant concentration ranges without involving sophisticated instrumentation, illustrating the applicability for on-site drinking water monitoring.

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1. Introduction

Metal ions play an important role in living organisms since they are involved in many biological processes such as osmotic regulation, catalysis, metabolism, biomineralization and signaling [1]. However, abnormal concentration levels of metal ions might cause detrimental effects, for example, trivalent aluminum (Al³⁺) in

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aqueous solution has been reported to be highly toxic; main source of which is from food and drinking water. The maximum acceptable Al^{3+} concentration in drinking water is $200 \mu\text{g L}^{-1}$ ($7 \mu\text{M}$) according to European Economic Community (EEC) standards [2]. Excess amount of Al^{3+} is believed to cause brain diseases such as dementia dialitica, Parkinson's disease and Alzheimer's disease [3,4]. The poisoning risk could also exist with the accumulation of lower level Al^{3+} in the brain [5]. Therefore developing a method to sensitively and selectively detect Al^{3+} is crucial. In the recent years, various methodologies for metal ion detection have been reported based on potentiometric [6–8], colorimetric [9–11], fluorescence [12–14], liquid chromatography [15], inductively coupled plasma-atomic emission spectrometry (ICP-AES) [16], atomic absorption spectrometry (AAS) [17], etc. Among the reported methodologies, colorimetric sensors have attracted great attention since they are facile and do not involve sophisticated instrumentation. Colorimetric sensors therefore have been investigated for Al^{3+} detection [18–23]. Most reported methodologies demonstrated an evident visual difference only under UV light and their inability to detect the Al^{3+} in aqueous solution limited their practical applications. Herein, we propose a colorimetric Al^{3+} sensor for both naked eye and fluorescence detection based on water-soluble conjugated copolymers.

Conjugated polymers are macromolecules consisting of alternating single/double bonds and delocalized π electrons throughout their backbones. This unique molecular structure of conjugated polymers offers attractive optical and electrical properties. Thus conjugated polymers, for instance, poly(phenylene vinylene) (PPV) [24], poly(phenylene ethylene) (PPE) [25], polydiacetylene (PDA) [26], polythiophene (PT) [27,28] and polyfluorene (PF) [29] have been widely reported as a colorimetric or fluorescence chemosensors. However, most reported conjugated polymers exhibit rather poor solubility in water probably due to their low charge densities which compete with the aromatic π - π stacking of the hydrophobic backbones [30]. This limits their applicability for biomolecular detection, water quality monitoring, etc. More

recently, cationic PTs and their derivatives have received much attention due to their good water solubility and attractive optical properties. Electrostatic force-induced conformational change in PT backbone often leads to color as well as the fluorescence intensity changes. Adenosine triphosphate (ATP) is one of the reported anionic molecules that induced significant color and fluorescence intensity change upon non-covalent bonding with cationic PTs and their derivatives [31]. However, electrostatic complex of homopolymer PTs and ATP may not be ideal for detection of trivalent metal ions due to either irreversible complexation and/or weak interaction. Therefore, a rationally designed and synthesized PT random copolymer is utilized in this study for complexation with ATP and subsequently for selective and sensitive detection of Al^{3+} .

The synthesized PT random copolymer was incubated with negatively charged ATP to form a complex based on the electrostatic self-assembly [32,33]. The color of resulting solution changed immediately (from yellow to purple), and the fluorescence intensity was quenched to around 15% of its original intensity. Addition of Al^{3+} resulted in the recovery of color (from purple to yellow) and fluorescence intensity due to the disassembly of PT-ATP complex as a result of strong affinity between Al^{3+} and ATP [34]. The formation of Al^{3+} -ATP complex triggered the transformation from a planar π -stacking morphology of PT copolymer to a random coiled morphology, thus resulting in recovery of color and fluorescence of PT copolymer. The proposed methodology enabled selective and sensitive detection of Al^{3+} without sophisticated instrumentation. We furthermore demonstrate similar responses to Al^{3+} in tap water, illustrating the applicability for drinking water quality monitoring, as water from the tap is typically used for drinking either as it is or upon boiling.

2. Experiment section

A novel water soluble PT monomer (m1, m2) and copolymer (CP) were synthesized adopting the protocol reported previously [35–37]. The structure of monomers (m1, m2) and CP is shown in

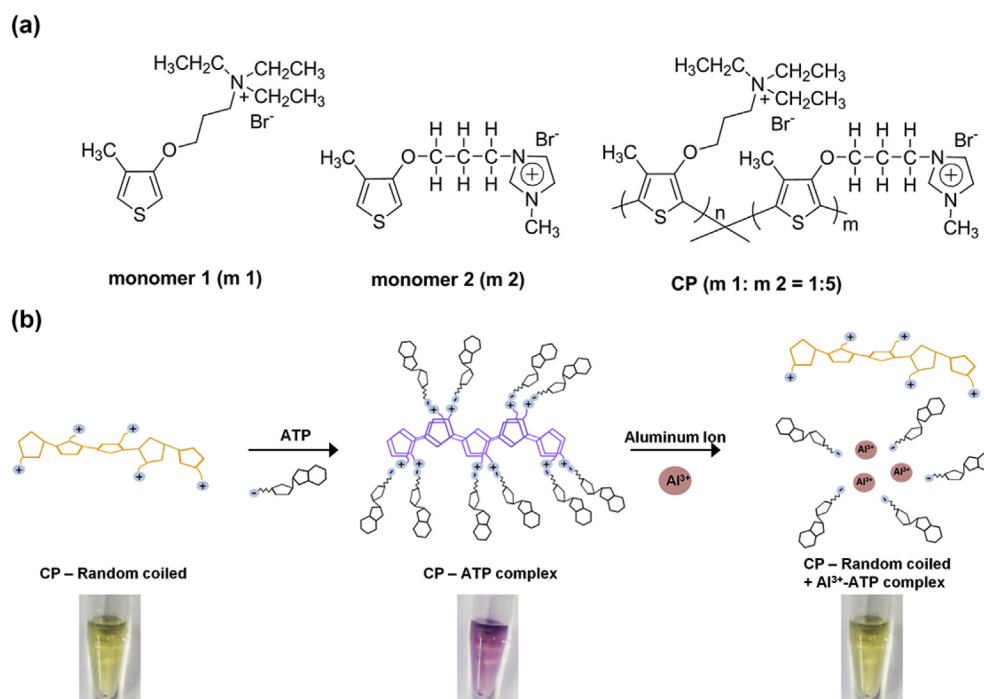


Fig. 1. (a) Structure of monomer 1, monomer 2, and its copolymer (CP). (b) principle of Al^{3+} detection. For clarity, the substituted groups are not shown.

Fig. 1a. The protocol adopted for synthesis of m1, m2 and CP and their corresponding NMR spectrum are provided in supplementary information (Fig. S1–S3). For selective Al^{3+} detection, two processes were evaluated; (1) CP was incubated with ATP first, followed by addition of Al^{3+} ; (2) Al^{3+} was incubated with ATP, followed by addition of CP. In order to develop the colorimetric sensor, method 1 was utilized for all experiments in this article, yielding colorimetric responses upon addition of Al^{3+} . Method 2 was used to investigate the mechanism of interaction between Al^{3+} and ATP. CP solution was stored in 4 °C fridge while not in use. Lithium chloride and Cobalt (II) chloride were purchased from Alfa Aesar. The other metal chlorides were purchased from Sigma Aldrich and used without further purification. The measurement of fluorescence and UV–Vis spectrum were conducted by microplate readers (infinite m200 pro, TECAN).

3. Results and discussion

An optimized ratio of monomers (m1:m2 = 1:5) for synthesis of CP was utilized based on the colorimetric response and Al^{3+} ion selectivity of PT1 and PT2, which are the homopolymers of monomer 1 and monomer 2, respectively. As shown in Fig. S4, PT1 demonstrated more evident colorimetric response to metal ions

than PT2, while the selectivity is relatively poor with respect to Cr^{3+} , Fe^{2+} , Pb^{2+} (400 μM). PT2, however, showed a good selectivity to Al^{3+} among all ions tested, but, the colorimetric response was not significant for naked eye detection, especially at low concentration levels of metal ions. The experimental results therefore suggest that a combination of monomers 1 and 2 could be utilized for selective and sensitive detection of Al^{3+} ions. Of the monomer compositions tested, a stoichiometric ratio of m1:m2 = 1:5 was utilized in order to maximize colorimetric response while retaining selectivity.

Fig. 1b demonstrates the scheme of assay development; the color of random coiled CP solution (80 μM) changed (from yellow to purple) immediately upon the addition of 16 μM ATP due to an electrostatic complexation of CP-ATP. This complexation promotes planarization of the CP backbone, and above a critical concentration efficient π - π stacking interaction between CP backbones is induced by hydrophobic interaction [31]. The color recovered upon addition of Al^{3+} due to disassembly of the CP-ATP complex and formation of more energetically favorable Al^{3+} -ATP complex. Al^{3+} was stabilized by Al^{3+} -phosphate coordination thereby inducing disassembly of CP-ATP complex to random coiled CP. The assay was tested in various concentrations of Al^{3+} (from 40 nM to 400 μM) using fluorescence and UV–Vis spectroscopy.

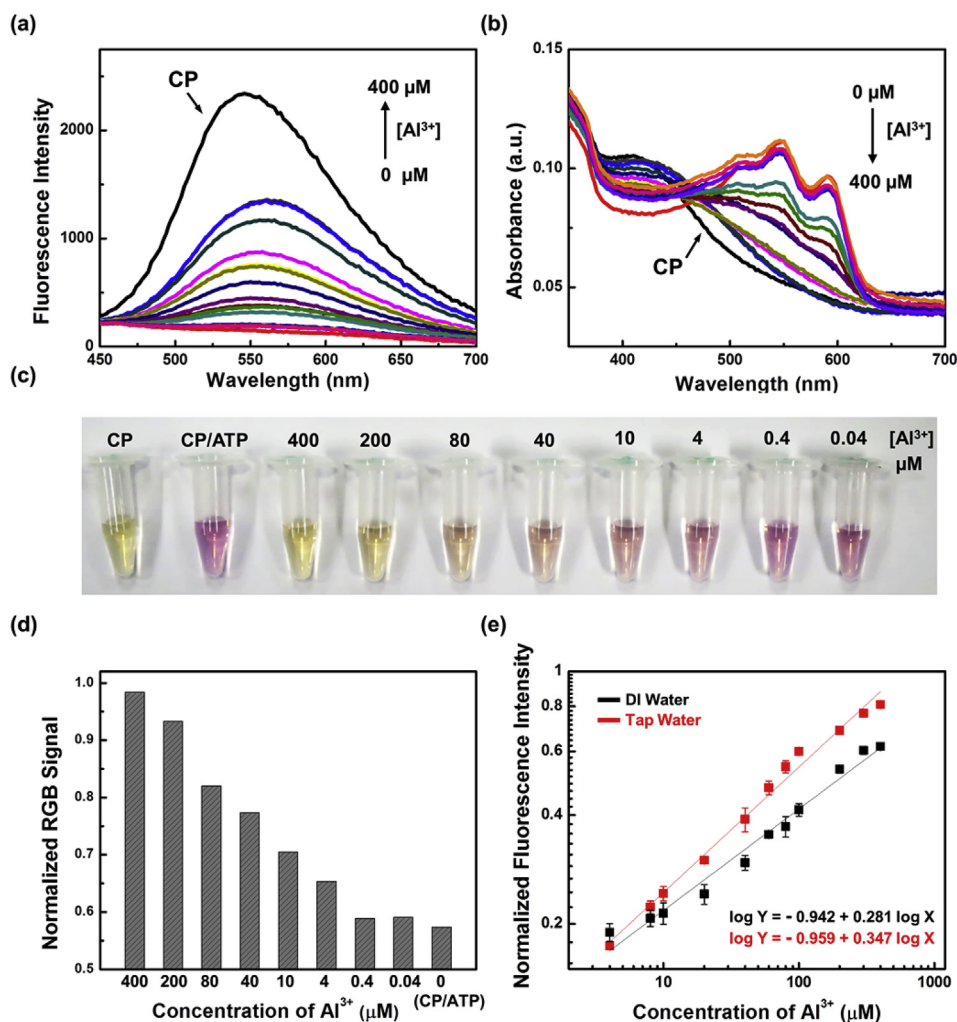


Fig. 2. (a) Evaluation of fluorescence spectra, (b) UV–Vis spectra, and (c) color change upon addition of various concentrations of Al^{3+} . (d) RGB analysis of Fig. 2c. (e) Correlation curve between normalized fluorescence intensity and concentration of Al^{3+} in DI water (black line) and tap water (red line), $n = 3$ for each Al^{3+} concentration. Concentration of CP: 80 μM ; Concentration of ATP: 16 μM .

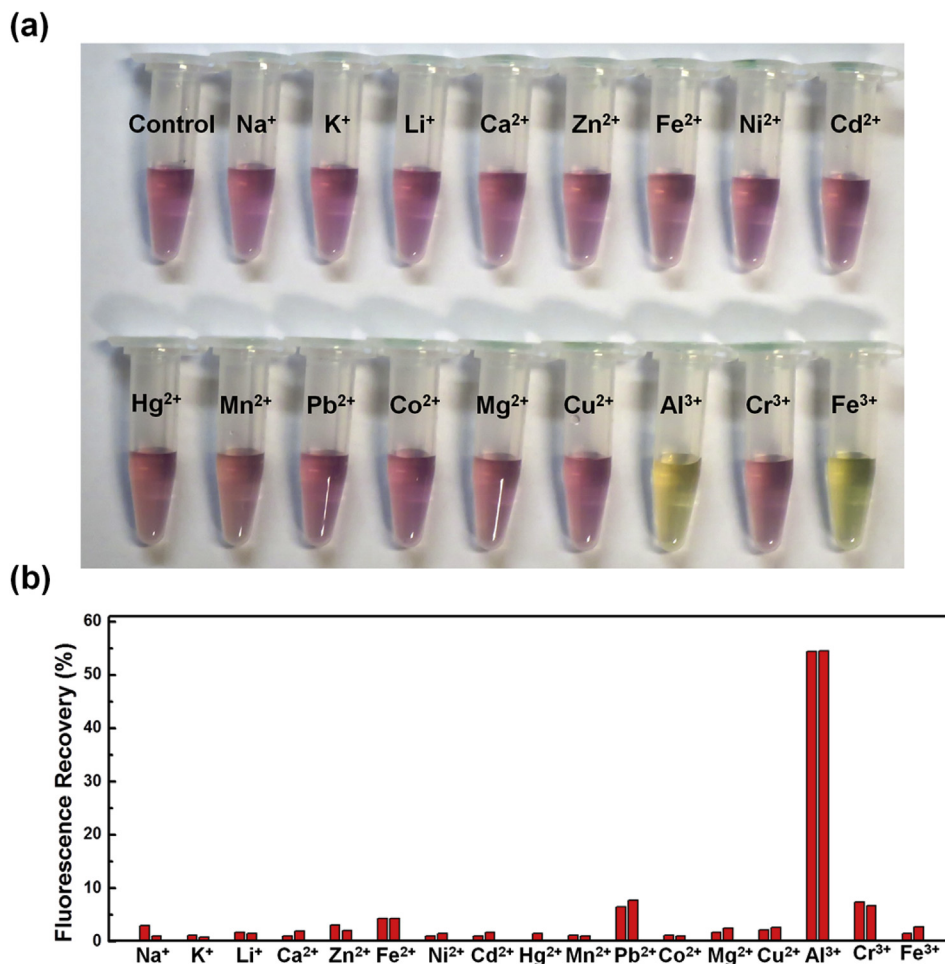


Fig. 3. (a) Color images and (b) fluorescence recovery upon addition of various metal ions (400 μM for each metal ions), left bar indicated CP incubated with ATP first, while right bar represented metal ion incubated with ATP first (according to methods 1 and 2, respectively).

The fluorescence intensity of solution containing 80 μM CP and 16 μM ATP in deionized (DI) water gradually increases with increasing concentration of Al^{3+} (Fig. 2a), which is attributed to the substitution of CP in CP-ATP complex with Al^{3+} , resulting in formation of ATP- Al^{3+} complexes. In the UV-Vis spectrum analysis (Fig. 2b), the peak intensity around 400 nm, associated with random coiled CP morphology increases with increasing concentrations of Al^{3+} whereas the vibronic peaks at 544 and 590 nm associated with CP-ATP planar π -stacking morphology decreases in intensity with increasing concentration Al^{3+} . Fig. 2c shows the optical image of CP-ATP vials incubated with various concentrations of Al^{3+} (40 nM–400 μM , from right to left), recorded using a digital camera. As observed from Fig. 2c, visually, 400 μM Al^{3+} almost fully recovered the color. The lowest concentration of Al^{3+} for naked eye detection was around 4 μM , which is below the Al^{3+} maximum acceptable concentration in drinking water according to the EEC standards [2]. Furthermore, the proposed approach ascertained detection of Al^{3+} ions at sub μM levels via a simple RGB analysis. RGB analysis has been reported as an efficient tool to quantify colorimetric response [27,38–40]. The RGB analysis was performed via software ImageJ, where 2000 pixels of each Al^{3+} concentration were chosen to read the average RGB value. The normalized RGB signal V/V_{CP} , where $V = G_{\text{value}}/B_{\text{value}}$ is shown on Fig. 2d (RGB values are taken from Fig. S5). As shown in Fig. 2d, the RGB result is in agreement with naked eye observation, suggesting that the proposed approach could be used in combination with

ubiquitous image capturing and processing devices such as digital cameras and smart phones, for highly sensitive detection of Al^{3+} ions.

We have also correlated the normalized fluorescence intensity derived from I/I_{CP} , where I and I_{CP} represent integrated fluorescence intensity from 450 to 700 nm for tested assay and CP, respectively, to the concentration of Al^{3+} ions in DI and tap water ($n = 3$ for each Al^{3+} concentration). As shown in Fig. 2e, the assay yielded a linear response on a log-scale with a corresponding regression equation: $\log Y = -0.942 + 0.281 \log X$, and the dynamic range of Al^{3+} detection was from 4 μM to 400 μM . The limit of detection (LOD) was estimated to be 3.7 μM ($S/N = 3$). In order to evaluate the sensor for practical applications, various concentration of Al^{3+} was spiked in tap water (Jurong, Singapore). As shown in Fig. 2e, the curve profile of tap water was similar to that of DI water. However, the normalized fluorescence intensity of assay in tap water was higher than the assay in DI water, which is attributed to the lower fluorescence intensity of I_{CP} . A possible explanation is that there were other anions existing in tap water compared to DI water and the electrostatic force of those anions contributed to formation of planar π -stacking phase, further lowering the fluorescence intensity of CP (I_{CP}). Al^{3+} addition recovered the fluorescence not only from the CP-ATP, but also from CP-anions, thus the normalized fluorescence intensity was higher in tap water than in DI water. The regression equation of Al^{3+} assay in tap water was $\log Y = -0.959 + 0.347 \log X$, and the LOD was calculated to be

Table 1
Comparison of this strategy with other Al³⁺ colorimetric/fluorescence assays.

Methodology	Pure aqueous	Target metal ion	Limit of detection (LOD)	Tap water test	Ref.
Colorimetric and fluorescence assay	x	Cr ³⁺ , Al ³⁺	Cr ³⁺ : 0.2 μM Al ³⁺ : 0.5 μM	x	[18]
Fluorescence assay	x	Al ³⁺	0.37 μM	x	[19]
Colorimetric assay	✓	Al ³⁺	DI water: 1 μM Tap water: 5 μM	✓	[20]
Fluorescence assay	x	Al ³⁺	1 μM	x	[21]
Fluorescence assay	✓	Al ³⁺	3 μM	x	[22]
Fluorescence assay	x	Al ³⁺	0.1 μM	x	[23]
Colorimetric and fluorescence assay	✓	Al ³⁺	DI water: 3.7 μM Tap water: 3.6 μM	✓	Reported methodology in this work

"x" and "✓" refer to "not validated" and "validated" for mentioned test conditions, respectively.

3.6 μM (S/N = 3), which is comparable to the estimated LODs obtained using naked eye and RGB detection strategies.

Fig. 3a illustrates the selectivity of CP-ATP complex toward Al³⁺ as compared to other metal ions tested (at 400 μM). The image indicates color recovery (to original yellow color) upon addition of Al³⁺, whereas addition of 400 μM of other metal ions except Fe³⁺ yielded almost negligible recovery, indicating again minimum interferences. Besides, the fluorescence recovery ability of Al³⁺ under other ions interference (400 μM for each metal ion) was tested as shown in Fig. S6, only Hg²⁺ shows a slight interference to Al³⁺. However, it should be noted that 400 μM is several orders higher than normal levels of Hg²⁺ in drinking water (~10 nM) and rarely found in drinking water even in mercury poisoning incident (Table S1). The adopted approach, however, illustrates that the assay might respond to extremely high concentration of interfering ions. For such demanding applications, a possible methodology to selectively evaluate Al³⁺ response is shown in Fig. S7. In case of Fe³⁺ in Fig. 3a, although the color turned to yellow, the mechanism is not same as Al³⁺, as it is quenching the fluorescence of CP instead of recovering it as shown in Fig. S8. In addition, the colorimetric responses of CP in the presence of other metal ions commonly found in drinking water (Fig. S9) shows that the proposed assay is applicable for selective detection of Al³⁺, despite the presence of high concentration of interfering ions (400 μM). The sensor response is also in agreement with previous reports on strong affinity between Al³⁺ and ATP, leading to the formation of ATP-Al³⁺ complex that liberate CP's occupied positive sites and causing recovery of CP fluorescence [34]. Fig. 3b shows the fluorescence recovery for 17 metal ions, where the fluorescence recovery % is defined as $(I - I_{ATP}) / (I_{CP} - I_{ATP})$, where I_{ATP} represents integrated fluorescence intensity of CP-ATP complex.

In order to investigate the affinity between ATP and Al³⁺, the process steps in method 2 (as mentioned in experimental section) was adopted, where ATP was incubated with Al³⁺ followed by addition of CP. Higher fluorescence intensities were recorded, probably due to the complexation of Al³⁺ with ATP, thereby decreasing the amount of free ATP in the solution, leading to less fluorescence quenching upon CP-ATP electrostatic interactions. This observation is consistent with Fig. S10 (UV-Vis data). The absorbance ratio between 400 nm and 544 nm was used to estimate the amount of random coiled and planar π -stacked CPs. Although the resulting signal was slightly higher in method 2, method 1 was chosen as the main procedure for the colorimetric assay since the visual color changes was more pronounced upon the addition of Al³⁺ in method 1. Method 2 was merely tested as an alternative way to study the affinity between ATP and Al³⁺. Table 1 shows the comparison of this work and other Al³⁺ assays reported in literature with comparable LOD in pure aqueous solution. Besides, results in tap water show a similar response compared to DI water, indicating this assay is highly selective to Al³⁺ from other

common ions in tap water.

4. Conclusions

A colorimetric and fluorescence assay based on water soluble conjugated copolymer for Al³⁺ detection was successfully developed. The electrostatic assembly-disassembly between CP and ATP provides evident color difference for naked eye observation. The lowest concentration for naked eye observation and RGB analysis of Al³⁺ is around 4 μM, which is below the stipulated threshold levels in drinking water, according to EEC standards. Besides, the proposed assay shows negligible interferences with the existence of other high concentration metal ion or in tap water environment, indicating this methodology could be used in selective and sensitive detection for Al³⁺ in analytically relevant concentration ranges without involving sophisticated instrumentation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.aca.2015.12.002>.

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