A Chalcone Based Novel Fluorescent Nanoprobe for Selective Detection of Al$^{3+}$ Ion in Aqueous Medium

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Abstract

A chalcone based novel fluorescent organic nanoparticles are prepared by reprecipitation method. The Dynamic Light Scattering histogram shows narrow particle size distribution and the average diameter of particle is 37.2 nm. The Scanning Electron Microphotograph shows the fibre like morphology. The blue shifted UV-visible spectra. The significant large Stokes shift of Trans-3-(9-Anthryl)-1-phenylprop-2-en-1-one nanoparticles (APPONPs) in aqueous suspension as compared with that of a dilute solution of Trans-3-(9-Anthryl)-1-phenylprop-2-en-1-one (APPO) in acetone indicates H- aggregation of molecules by π-π stacking. An intense fluorescence band peaking at 556 nm is attributed to Aggregation Induced Enhanced Emission (AIEE) by direct excitation of APPONPs. The binding of Al$^{3+}$ increased the fluorescence of APPONPs while other cations actually decreases the fluorescence. The fluorescence enhancement of APPONPs by Al$^{3+}$ was discussed on the basis of hard and soft acid and base (HSAB) theory. The enhancement of APPONPs emission is linear in the concentration range 0-80 μM of Al$^{3+}$. The enhancement of fluorescence of APPONPs results obeys the straight line equation with RSD = 0.9879%. The present method has Limit of Detection (LOD)= 1.148 μM of Al$^{3+}$ which is lower than the LOD reported for Al$^{3+}$ by using other methods like colorimetry, potentiometry. This study was successfully applied to develop fluorimetric method for determination of Al$^{3+}$ in environmental samples. The method affords fairly practical and economical approach.

Keywords: Chalcone; Aggregation Induced Enhanced Emission (AIEE); Organic nanoparticles; Fluorescence enhancement; Al$^{3+}$ detection; Ground state Complexation

1. Introduction

The multifunctional activities through organic molecules to form nanoparticles have been a topic of considerable interest. The preparation of Fluorescent Organic Nanoparticles (FONs) and their application as an analytical probe to detect metal ions, cations, anions, biomolecules is an enormously active area of research due to their remarkable optical and electronic properties.
Aluminum is the third most prevalent element and the most abundant metal in the earth’s crust. However the unregulated amount of aluminum to human body may lead to the malfunction of central nervous system, Parkinson’s and Alzheimer’s diseases. Apart from these, the toxicity of aluminum endangers the aquatic life and influences agricultural production in acidic soils (Azadbakht R, et al. 2013, Singh D P and Singh V P, 2014, Xiaobo L, et al. 2014). The high Al$^{3+}$ content affects the growth of plants. Thus the detection of Al$^{3+}$ in biological, pharmaceutical and environmentally important samples is a significant issue to address. Known methods for Al$^{3+}$ ion determination in aqueous medium, such as potentiometric and colorimetric method, inductively coupled plasma spectrometry and atomic absorption spectrometry, are tedious and expensive in practice. Comparatively, optical detection, particularly spectrofluorimetric methods, shows unique potential with high sensitivity and selectivity towards metal ion detection (Azadbakht R, et al. 2013, Saleh M B, et al. 2001, Yang N, et al. 2014). The chalcones and their derivatives are important intermediates in organic synthesis (Patel U, et al. 2013). They serve as starting material for the synthesis of a variety of heterocyclic compounds which are of physiological importance. Due to the presence of enone functionality in chalcone moiety confers biological activity upon it, like anti-inflammatory (Ballesteros J, et al. 1995), antimalarial (Liu M, et al. 2003), antituberculosis (Sivakumar P, et al. 2007), anticancer (Syam S, et al. 2012) and antivascular activities (Ducki S, et al. 2009). Chalcone derivatives or chalcone has keto functionality may interact with cation of particular interest. To the best of our knowledge up to date, no reports are available on the chalcone derivatives used as a fluorescence nano probe to detect cation, anion, biomolecules, etc. Consequently it is proposed to prepare the functionalized organic nanoparticles using chalcone derivative to enable as probe for detection of particular ion in the desired samples. Present paper reports the studies on preparation of Trans-3-(9-Anthryl)-1-phenylprop-2-en-1-one nanoparticles (APPONPs) and the comparative smaller radius and high charge density of Al$^{3+}$ probably favors its coordination with carbonyl functionality containing APPONPs produce fluorescence changes required to explore potential of nanoparticles as fluorescence probe.
2. Experimental

2.1 Materials and Instrumentations

The analytical grade reagents and chemicals required in the studies were obtained from the commercial sources. 9-Anthraldehyde, acetophenone, KOH and Metal salts of all cations were procured from Sigma Aldrich (India). Acetone and ethanol were purchased from Alfa Aesar (Johnson Matthey, India) and were used as solvents after distillation. Millipore water (Millipore, India) was used during the experiments. (Millipore water of pH= 6.87 (kept constant at 7 by using phosphate buffer), Resistivity = less than 18.2 MΩ. cm, Content = < 5 ppb total organic carbon).

2.2 Instrumentations

$^1$H (300 MHz) and $^{13}$C (75 MHz) NMR spectra were recorded in CDCl$_3$ on Bruker Avance 300 NMR spectrophotometer. Chemical shifts were reported using tetramethylsilane (TMS) as an internal standard. Infrared spectrum was recorded in KBr on a Perkin Elmer FT-IR spectrophotometer in the 4000–400 cm$^{-1}$ region. Mass spectrum was recorded on Shimadzu QP 2010 GCMS. UV–visible spectra and Fluorescence spectra of the solutions were recorded on a Shimadzu spectrophotometer and on a JASCO FP-8300 spectrofluorometer, respectively. The size and zeta potential of the APPONPs was measured using a Malvern Zetasizer (nano ZS-90) equipped with a 4 mW, 633 nm He–Ne Laser (U.K.) at 25°C under the fixed angle of 90° in disposable polystyrene cuvettes. The morphology of APPONPs was assessed by Scanning Electron Microscope (SEM), (JEON-6360 Japan), operated at an accelerating voltage of 20 kV. The pH of the solution of nanoparticles was measured on LI120 digital pH meter.

2.3 Procedure

2.3.1 Synthesis of Trans-3-(9-Anthryl)-1-phenylprop-2-en-1-one: (APPO)

Claisen-Schmidt condensation (Choudhary A N and Juyal V, 2011, Suwito H, et al. 2014) reaction is the basis of the synthesis of Chalcone derivative (APPO) and route is shown in Scheme 1. A mixture of 9-anthraldehyde (2.06g, 0.01 mol), acetophenone (1.22 g, 0.01 mol) and potassium hydroxide pellets (0.90 g, 0.015 mol) in ethanol (30 ml.) was stirred at 60 °C for 12 h. Later the reaction mixture was kept in refrigerator for 1h. The completion of reaction was confirmed by TLC in Pet ether-Ethyl acetate (80:20) system. The solid
product precipitated out was filtered and recrystallized from hot ethanol. The final product APPO is yellow colored and is of appreciable yield.

### 2.3.2 Spectral Characteristics of Compound

The formation of APPO was confirmed by spectral techniques viz. IR, $^1$H, $^{13}$C NMR and MS. (87.9%, Practical yield); MP 120 °C (Literature): 119 0 C (Observed); IR (KBr pallet, Fig. S1, ESǂ): 1659 cm$^{-1}$, α-β unsaturated carbonyl group; $^1$H NMR (300 MHz , CDC13, Fig.S2. ESǂ): δ 7.52–8.51 (14 H, m, aromatic), δ 8.857 (1 H, dd, J=15.9Hz, Vinylc), δ 8.351 (1H, dd, J=15 Hz, Vinylc); $^{13}$C NMR (75 MHz, CDC13, Fig.S3. ESǂ): C= 0, δ 189.72; C= C, δ 141.94  and δ 137.90; Ar-C, δ 125.08-δ 133.13; MS (EI) (Fig.S4. ESǂ): 308 (M$^+$), 203, 105 m/z.

### 2.3.3 Preparation of Trans-3-(9-Anthryl)-1-phenylprop-2-en-1-one nanoparticles: (APPONPs)

Nanoparticles of APPO in aqueous suspension was prepared by the reprecipitation method (Bhopate D P, et al. 2013, Dalavi D K, et al. 2014, Mahajan P G, et al. 2015, a, b). A solution of APPO in acetone ($1 \times 10^{-4}$ mol L$^{-1}$, 5ml) was purged into 100 ml water and stirred vigorous by using magnetic stirring for 1h and then sonicated for 10 min. The content was allowed to stand for 30 minutes for stabilization. The polar APPO molecules nucleates and self assembled in aggregates to give nanoparticles in aqueous suspension.

### 3. Result and Discussion

#### 3.1 Characterization and Photophysical properties of APPONPs

The surface morphology and particle size distribution with zeta potential of APPONPs examined by Scanning Electron Microscope (SEM) and Dynamic Light Scattering (DLS) with zeta sizer equipments was discussed and given as supplementary information (Fig. S5., S6, S7 ESǂ). The photophysical properties of APPONPs in aqueous suspension studied by UV-Visible absorption and Fluorescence spectroscopy was also given as supplementary information (Fig S8. and S9. ESǂ). The blue shift is seen in absorption spectrum with respect to absorption maximum of sample molecule is shown to occur by possible H-aggregates and enhanced emission of nanoparticles is explained on the basis of restricted vibration motion due to molecular aggregation. The characterization and spectral results given as supplementary information are published elsewhere (Mahajan P G, et al. 2015, b).

#### 3.2 Effect of pH

The intensity of AIEE (Lim S, et al. 2004, Zhang X, et al. 2013) of surfactant free APPONPs was affected by pH changes of an aqueous suspension. It is well known that chalcones are pH-sensitive, so it is important to investigate the effect of pH on the performance of the nano probe. In the present study, the effect of the pH of the solution on the fluorescence intensity was studied and the results are shown in Fig. 1. Carmody buffer were tested in the pH 1.0-12 range and the results show that a pH of 7.0 was the best. The optimum range of pH is 6.0–8.0. The fluorescence intensity decreases when the pH is too low or too high. At low pH (acidic) and high pH (basic), the carbonyl and alkene group of chalcone moiety might be interacting with the free hydroxyl groups of citric and boric acid (carmody buffer components) through hydrogen bonding by which APPONPs disaggregates and its emission is reduced [Mahajan P G, et al. 2015, a]. The maximum fluorescence

intensity seen at pH=7.0 (Neutral) indicates high power stability of nanoparticles at this pH. Therefore, in the present work pH 7.0 with phosphate buffer as the buffer solution was chosen throughout the experiment.

![Fig. 1. Effect of pH of fluorescence intensity of APPONPs](image)

### 3.3 Recognition and Selectivity of APPONPs to Al^{3+}

The negative zeta potential -14.3 mV of APPONPs in aqueous suspension indicates its ability to bind cations. The binding ability was examined by measuring changes produced in the fluorescence spectrum of APPONPs after addition of aqueous solutions of metal ion prepared from their respective metal salts. Fig. 2. shows fluorescence spectra of aqueous suspension of APPONPs recorded in the presence of metal ions viz. Al^{3+}, Na^{+}, K^{+}, Ni^{2+}, Cu^{2+}, Pd^{2+}, Hg^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+}, Mg^{2+}, Pb^{2+}, Mn^{2+} and Fe^{3+} of concentration 40 µM each. It is seen that presence of Al^{3+} exhibit fluorescence enhancement while other cations responds oppositely and seen to quench the fluorescence of APPONPs.

![Fig.2. Selective fluorescence enhancement of APPONPs by Al^{3+}](image)
The bar diagram shown in Fig. 3 reveals the fluorescence intensity enhancement as ratio of $\frac{\Delta F}{F_0}$, where $\Delta F = F - F_0$, $F_0$ and $F$ are the fluorescence intensity of nanoparticle in absence and presence of coexisting cations measured at wavelength 556 nm. It is seen that the increase in fluorescence intensity is significant only for Al$^{3+}$ cation (Fig. 3, Blue bar) whereas other cations do not respond to produce fluorescence enhancement rather showed fluorescence quenching. The complexation of APPONPs with Al$^{3+}$ increases the rigidity of the molecular assembly by restricted free rotations of the carbonyl group with respect to the anthracene and the phenyl ring results in a significant enhancement of the fluorescence intensity. Other cations due to fluorescence quenching, do not cause interference in detection of Al$^{3+}$ in aqueous solution (Fig. 3, Red bar). Fig. 4 indicates the selective fluorescence enhancement observed only for Al$^{3+}$ ion in presence of more than one or several coexisting cation simultaneously. The presence of more than one or several such metal ion does not interfere in the Al$^{3+}$ ion detection in aqueous solution using APPONPs as fluorescent nanoprobe. The selectivity of APPONPs towards Al$^{3+}$ may be explained on the basis of smaller ionic radii (0.5Å) and higher charge density ($r = 4.81$) of the Al$^{3+}$ ion. The smaller radius of Al$^{3+}$ ion allows appropriate co-ordination for the APPONPs and higher charge density allows strong ability between the receptor and Al$^{3+}$ ion (Liu Y, et al. 2012).

![Fig. 3](image-url)

**Fig. 3.** Fluorescence Intensity response [$\Delta F/F_0$] in the presence and Absence of the Al$^{3+}$ ion and several coexisting cations viz. Na$^+$, K$^+$, Ni$^{2+}$, Cu$^{2+}$, Pd$^{2+}$, Hg$^{2+}$, Cd$^{2+}$, Co$^{2+}$, Zn$^{2+}$, Mg$^{2+}$, Pd$^{2+}$, Mn$^{2+}$ and Fe$^{3+}$. Solutions of 40 µM concentrations monitored at excitation wavelength 298 nm.

The fluorescence enhancement effect was further understood from the fluorescence titration results of APPONPs against Al$^{3+}$ ion solution given in Fig. 5. The incremental addition of Al$^{3+}$ ion in an aqueous suspension of the APPONPs, produce the fluorescence response immediately and fluorescence intensity is seen to increase up to 80 µM added solution of Al$^{3+}$. The results fit into the straight line equation as shown in Fig. 6.
Fig. 4. Effect on fluorescence intensity of APPONPs in presence of Al$^{3+}$-ion and more than one or several coexisting ion solution of 40 µM concentrations monitored at excitation wavelength 298 nm.

Fig. 5. Fluorescence Spectra of aqueous suspension of APPONPs in the presence of increasing concentrations of Al$^{3+}$ ions (00, 10, 20, 30, 40, 50, 60, 70, 80 µM).

The detection limit was calculated according to the IUPAC definition (Bhopate D P, et al. 2013, Mahajan P G, et al. 2015, a) using equation 1 in the linear range from 0 to 80 µM and found to be 1.148 µM which was lower as compared to other methods reported so far (Dhara A, et al. 2013, Ding P, et al. 2015, Kumar J, et al. 2015, Saleh M B, et al. 2001, Yang N, et al. 2014).

$$LOD = \frac{3.3\sigma}{K} \quad \text{------------------------------------- (1)}$$
where, $K$ is the slope of graph and $\sigma$ is the standard deviation of y-intercept of regression lines.

**Fig. 6.** $\Delta F$ as function of concentration of $\text{Al}^{3+}$ ion solution

### 3.5 Mechanism of binding APPONPs and $\text{Al}^{3+}$

The binding mode between APPONPs and $\text{Al}^{3+}$ ions was further investigated by examining the UV-visible spectra of APPONPs recorded in the absence and in the presence of various concentrations of $\text{Al}^{3+}$ shown in Fig. 7. The intense sharp absorption band of APPONPs appearing at 252 nm seen disappeared and new broad emission band appeared in the region 275 to 330 nm. The absorption intensity of this band increases with $\text{Al}^{3+}$ ion concentration. The observed new red shifted absorption band suggests the possibility of ground state complexation between APPONPs and $\text{Al}^{3+}$ ion.

**Fig. 7.** Absorption spectra of APPONPs in aqueous suspension in presence of $\text{Al}^{3+}$ ion solution of concentration in the range from 10 µM to 80 µM
Fluorescence enhancement of APPONPs after addition of Al$^{3+}$ involves electrostatic interaction between oppositely charged nanoparticles and Al$^{3+}$ ion. In general, as Al$^{3+}$ is a hard acid, it has been found that Al$^{3+}$ prefers a coordination sphere containing nitrogen (N) and oxygen (O) which are hard- base donor sites (Li T, et al. 2014). The present sensor APPONPs containing oxygen donor atom display higher affinity to Al$^{3+}$ than the other test metal ions because of the much stronger Lewis acid character of Al$^{3+}$, the Al- O bond is formed because of a more ionic character (Xiaobo L, et al. 2014). The complex formation between APPONPs and Al$^{3+}$ ions increases the rigidity of the molecular assembly where by restricted free rotations of the carbonyl carbon with respect to anthracene and the phenyl rings results in a significant enhancement of the fluorescence intensity (Singh D P and Singh V P, 2014). The proposed binding mechanism involves strong interaction between the carbonyl group (oxygen end) and Al$^{3+}$ ion in aqueous medium. Fluorescence enhancement of nanoparticles on complexation with Al$^{3+}$ ion in the aqueous solution is further examined from the double logarithm plot of log $\Delta F$ against log [Al$^{3+}$] given as supplementary information (Fig. S10. ES†). The binding constant estimated from this plot is found to be $K = 9.927 \times 10^5$ M indicated high stability of complex and strong interaction. Possible mechanism of formation of nanoparticles and its interaction with Al$^{3+}$ ion is presented in Scheme 2.

![Scheme 2 Proposed mechanism of APPONPs formation and its interaction with Al$^{3+}$ ion](image)

### 4. Application

The fluorescence enhancement of APPONPs by Al$^{3+}$ was further used to develop an analytical method for quantitative determination of Al$^{3+}$ from water samples. The method includes determination of Al$^{3+}$ ions in water samples collected from local area of Shivaji University campus, Kolhapur (SUK), Maharashtra, India. For the better results and analysis of Al$^{3+}$ ion in the environmental samples via standard addition method, the collected samples were undergone pre-treatment for sample purification to avoid the interference of other impurities and matrix in the environmental samples. The samples collected were first filtered through Whatmann filter paper No. 41 paper to remove suspended matter and impurities, followed by boiling for 10 minutes to remove chlorine and dissolved gases. The collected environmental samples were spiked with standard Al$^{3+}$ ion solution of two concentration level and diluted within the working range. The sample was analyzed by the proposed fluorimetric method using standard addition method and calibration curve shown in Fig 6. The standard addition technique involves adding known amount of standard to one or more aliquots of processed sample solution, compensating for sample
constituents that enhances or depresses their analyte signal. For multi point, the fluorescence intensity difference of each solution from fluorescence intensity of nanoparticle without analyte determines and plotted on vertical axis of graph, with the concentration of known standards plotted on horizontal axis. The total amount of analyte \((\text{Al}^{3+})\) spiked in environmental samples were determined by using calibration curve shown in Fig. 6. Further, Fig. 4 shows effect of two or more than two interfering ion along with presence of \(\text{Al}^{3+}\) ion on fluorescence intensity of APPONPs. From the results of Fig. 3 and Fig. 4 it is conclude that eventhough the presence of other ion along with \(\text{Al}^{3+}\) ion in aqueous solution, a very little and negliglible change in the fluorescence intensity of APPONPs was observed which could not interfere in the analysis of \(\text{Al}^{3+}\). The kind and concentration of interfering ions in the collected environmental water samples couldn’t show interference in the the analysis of \(\text{Al}^{3+}\) and could be neglected and further analysis was done for the \(\text{Al}^{3+}\) ion in the environmental samples. The recovery of \(\text{Al}^{3+}\) ion added in environmental samples was calculated by considering the added concentration of \(\text{Al}^{3+}\) ion solution (e.g. 20 and 40 µM) as 100%. The results are summarized in Table 1. It can be seen that the value of \(\text{Al}^{3+}\) found in the two concentration level are identical with expected values. The recovery and relative standard deviation are very satisfactory.

Table 1 Determination of \(\text{Al}^{3+}\) in different environmental water samples

<table>
<thead>
<tr>
<th>Water Sample Studied</th>
<th>Amount of standard (\text{Al}^{3+}) ion added (µM)</th>
<th>Total (\text{Al}^{3+}) ion found (µM)</th>
<th>Recovery of (\text{Al}^{3+}) ions added (%)</th>
<th>RSD(%)</th>
<th>Relative error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rajaram Lake, Near SUK.</td>
<td>20</td>
<td>19.97</td>
<td>99.85</td>
<td>0.031</td>
<td>-0.0015</td>
</tr>
<tr>
<td>Tap Water (Department of Chemistry, SUK)</td>
<td>40</td>
<td>39.49</td>
<td>98.72</td>
<td>0.753</td>
<td>-0.0128</td>
</tr>
</tbody>
</table>

\(n\) : Average of three determinations

4. Conclusion

The fluorescent Trans-3-(9-Anthryl)-1-phenylprop-2-en-1-one nanoparticles (APPONPs) prepared by reprecipitation method are of narrower particle size distribution showed by DLS histogram. The SEM examination indicated fibre like morphology. The UV-visible absorption and fluorescence spectroscopy results indicated the H- bonded aggregates involving \(\pi\)-stacking and strong AIEE is arising due to hindered molecular rotations thus favoring radiative decay of nanoaggregates. The negative zeta potential and affinity of carbonyl group because of oxygen donor causes strong binding of APPONPs with oppositely charged \(\text{Al}^{3+}\) resulting into enhancement of fluorescence of nanoparticles. The fluorescence intensity result fits into the straight line equation with \(\text{RSD} = 0.9877\%\). The present method has lower Limit of Detection (LOD) = 1.148 µM for \(\text{Al}^{3+}\) as compared to other methods reported so far for \(\text{Al}^{3+}\). The present method was used to develop fluorimetric method for determination of \(\text{Al}^{3+}\) in environmental samples collected from local area. The method affords fairly practical and economical approach.
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