

Condensation Product of 5-Bromosalicylaldehyde and Amino Phenol: Fluorescence Sensor for Ascorbic Acid and AND Logic Gate

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Research Article

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Abstract

Condensation product of 5-bromosalicylaldehyde and aminophenol (**L**) has been synthesised and characterised. Fluorescence of **L** enhances by 23 times on interaction with Ce^{3+} while it is quenched completely by Ce^{4+} . Ascorbic acid (AA) is a well known strong reducing agent and this property has been used to act **L**: Ce^{4+} adduct as a fluorescence “on” sensor for AA. AA reduces Ce^{4+} into Ce^{3+} and thereby increasing fluorescence of **L** due to the formation of **L**: Ce^{3+} adduct. Molecules which generally coexist with AA viz. Cholesterol, Glucose, Sucrose and Dopamine found not to interfere. The interaction of **L** with Ce^{3+} , Ce^{4+} and subsequently with AA has been verified with cyclic voltammetry.

Introduction

Ascorbic acid (AA), commonly known as vitamin c, exists both in plants and animals. It has strong antioxidant property and takes part in a number of physiochemical and biochemical processes in human body. There is report that AA is associated with many chronic diseases like – gout, skin disease, infertility, mucositis, cancer and HIV/AIDS [1-3]. Due to strong antioxidant property AA can stop change in taste and smell in foods and beverages and therefore used in food, beverages, medical formula, cosmetics etc. [4]. AA is not synthesised in human body and need to be sufficiently supplemented through different food items in order to avoid many diseases. However excess intake of ascorbic acid may result health problems such as urinary stones, diarrhoea, stomach cramps etc. Hence determination of AA in different food items is of great importance.

Because of its importance, AA has been determined by a number of different methods like – UV/Visible spectroscopy [8,9], High Performance Liquid Chromatography (HPLC) [10], Electrochemistry [11-14] and Chemiluminescence [15]. But these methods are associated with drawbacks like – special sample preparation and high instrumentation cost. Fluorescence, as a technique, has advantages like - high sensitivity, simple sample preparation, relatively lower instrument cost, direct applicability to living cells and biological fluids [16-18].

There has been recent reports on fluorescence sensing of ascorbic acid but most of them are based on nanoparticles and quantum dots (QD). Green emission fluorescent based on silicon nanoparticle (SiNP) and bovine serum albumin templated MnO_2 nanosheets is reported. Initially the fluorescence of SiNP is quenched by MnO_2 but AA on interaction reduces MnO_2 into Mn^{2+} and SiNP gains back its fluorescence [19]. Fluorescent “on” sensor for the detection of AA has been developed based on the fluorescence resonance energy transfer (FRET) between graphene QDs and squaric acid (SQA)- Fe^{3+} [20]. MnO_2 nanosheets were interacted with MoS_2 QD to quench its fluorescence which is restored by interacting with AA due to reduction of MnO_2 [21]. Self assembly of 2,6-pyridine dicarboxylic acid sensitised Eu^{3+} and carbon dots results resometric fluorescence response towards AA [22]. The blue fluorescence of SiNP was quenched by $CoOOH$ nanoparticle and on interaction with AA the later nanoparticles are decomposed by redox process restoring the fluorescence of SiNP and hence facilitating

sensing of AA [24]. In another method the fluorescence of CD is decreased by Ag NPs produced in situ by interaction of AA with Ag(I) in presence of Ag NP seeds [25]. Graphene QD was complexed with dopamine by electrostatic interaction and H-bonding which was then coordinated with Cu^{2+} which quenched the fluorescence of QDs and the fluorescence quenching is removed by AA added to the solution resulting in AA sensing [26]. N doped CDs with high fluorescence quantum yields have been reported which could detect AA by fluorescence "off" mode due to inner filter effect [27]. In another reported method the fluorescence of N doped CD is quenched by Cr(VI) first, AA then reduces the Cr(VI) into Cr(III) restoring the fluorescence [28]. CdTe QDs have been reported to show red shift in fluorescence emission peak due to increase in its size on interaction with AA and hence acts as sensor for AA [29].

In this paper we report a very simple method for the detection of ascorbic acid where the fluorescence of the probe is first quenched by Ce^{4+} and then ascorbic acid reduced it into Ce^{3+} leading to very large increase in fluorescence intensity. The method is found to be interference free from cholesterol, glucose, sucrose and dopamine. The probe acts as AND logic gate for fluorescence output with Ce^{4+} and AA as input.

Experimental

Synthesis and characterization of sensor (**L**):

L was prepared by the method reported [30]. Briefly: an ethanolic solution of 1 mmol of 2-aminophenol (0.109 g) was added to the equimolar solution of 5-bromosalicylaldehyde (0.201 g) in absolute ethanol. The reaction mixture was stirred at room temperature for 07 hours, yellow coloured precipitate was obtained, collected by filtration.

All the chemicals were either from Merck or Loba Chemie. The metal salts except $\text{Pb}(\text{NO}_3)_2$, CaCl_2 and HgCl_2 were sulphates. Metal salt solutions were prepared in doubly distilled water obtained from quartz double distillation plant. The FT-IR spectra were recorded in a Perkin Elmer RXI spectrometer as KBr pellets, ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Ultra Shield 300 MHz spectrophotometer using DMSO D6 as solvent. The fluorescence and UV/Visible spectra were recorded in HITACHI 2700 and Shimadzu UV 1800 spectrophotometer respectively using quartz cuvette (1 cm path length). Electrochemical experiments were performed at CHI (USA) electrochemical analyser work station 660D. Pt disc was used as working electrode, Ag-AgCl (3 N NaCl) as reference electrode and Pt wire as auxiliary electrode. Nitrogen gas was passed at slow rate through the solution to remove dissolved oxygen.

Results And Discussion

L in 1:1 (v/v) $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (5×10^{-4} M) shows fluorescence spectrum on excitation with 270 nm photons in quartz cell of path length 1.0 cm. The emission was observed in 280 nm to 700 nm range with a maximum at 308 nm with intensity 360 (Fig. 1). Fig. 2 shows the fluorescence spectra of **L** in presence of one equivalent of different metal ions. From the figure it is clear that Ce^{3+} enhances fluorescence of

L significantly while Ce^{4+} quenches the fluorescence of **L**. Metal ions - Al^{3+} , Li^+ , Na^+ , Pb^{2+} , Cd^{2+} , Hg^{2+} , Mn^{2+} , Mg^{2+} , K^+ , Ca^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+} do not effect fluorescence spectra of **L**. Fig. 3 shows the fluorescence spectrum of **L** in 1:1 (v/v) $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ at different added concentration of Ce^{3+} . The I_{max} value for **L** was found to shift from 308 nm to 350 nm in presence of Ce^{3+} .

Fig. 4 shows the I/I_0 values where I_0 is the fluorescence intensity of **L** in absence of metal ions and I is the fluorescence intensity of **L** in presence of one equivalent of a particular metal ion in 1:1 (v/v) $\text{CH}_3\text{OH}:\text{H}_2\text{O}$. From the figure it is clear that Ce^{3+} could enhance the fluorescence intensity of **L** by 22 times while in case of other metal ions it is below 2 times and Ce^{4+} completely quenched the fluorescence intensity.

The fluorescence spectra of **L** in 1:1 (v/v) $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ at different added concentrations of Ce^{4+} is shown in Fig. 5. The 310 nm fluorescence peak initially shifts to 350 nm and then quenches with addition of Ce^{4+} with I_{max} shifting towards 370 nm before complete quenching of fluorescence intensity.

Fig. 6 shows the effect of fluorescence spectra of **L** in presence of one equivalent Ce^{4+} and on subsequent addition of AA. It is observed that fluorescence intensity increases with red shift in I_{max} on addition of AA and finally the I_{max} becomes 350 nm which is same to that of **L** in presence of one equivalent Ce^{3+} . AA is a strong reducing agent and therefore it reduces Ce^{4+} into Ce^{3+} and the fluorescence enhancement is observed. Fig. 7 shows the plot of fluorescence intensity as a function of AA concentration which is linear.

The interference by molecules generally present in biological fluids closely with AA viz. Cholesterol, Glucose, Sucrose and Dopamine have been investigated. For the purpose fluorescence of **L**, **L** + Ce^{4+} , **L** + Ce^{4+} + Interfering molecule + AA were recorded. Here concentration ratios for all the species have been kept as one equivalent. Fig. 8 compares the I/I_0 values through bars where I_0 is fluorescence intensity of **L** and I is the fluorescence intensity for **L** + Ce^{4+} + Interfering molecule + AA. The comparable height of the bars, in presence of AA, and I/I_0 values similar to that for **L** + Ce^{3+} (Fig. XX) confirms that Cholesterol, Glucose, Sucrose and Dopamine do not interfere detection of AA.

The effect of AA on **L** + Ce^{4+} + AA has been examined by UV/Visible spectroscopy also. Fig. 9 shows the UV/Visible spectrum of **L** + Ce^{4+} in presence of different added concentration of AA in 1:1 (v/v) $\text{CH}_3\text{OH}:\text{H}_2\text{O}$. In absence of AA peaks were observed at 284 nm and 422 nm for **L** + Ce^{4+} in 1:1 (v/v) $\text{CH}_3\text{OH}:\text{H}_2\text{O}$. On addition of AA the peak at 422 nm does not undergo any change while the absorbance of the peak at 284 nm increases gradually with a shift in I_{max} to 270 nm. Fig. 9, Inset shows the plot of absorbance versus AA concentration.

The interaction of **L** with Ce^{4+} and **L** with Ce^{4+} + AA has been verified by cyclic voltammetry at Pt working electrode using Ag-AgCl (3 M NaCl) in 1:1 (v/v) $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (Fig. 10). The green curve is for **L**, the red curve is for **L** + Ce^{4+} and the blue curve is for **L** + Ce^{4+} + AA. The cyclic voltammograms for **L** + Ce^{4+} is

found to be quite different from that for **L**. Addition of AA leads to a cyclic voltammogram which is similar to that for **L**. This confirms that the interaction between Ce^{4+} and **L** must be stronger than the interaction between Ce^{3+} and **L**.

The fact that Ce^{4+} quenches fluorescence of **L** while Ce^{3+} enhances it can be explained (Scheme 2) considering the ionic radii of Ce^{4+} and Ce^{3+} which are 115 pm and 101 pm respectively. Due to smaller size of Ce^{4+} it can fit into the hole created by ONO of **L** and bind to the two O of hydroxyl groups and the imine N of **L**. Due to its higher charge to size ratio Ce^{4+} withdraw the electron density from the conjugation system of **L** strongly and therefore the fluorescence is quenched. On the other hand Ce^{3+} being bigger do not fit into the hole and binds to the two O of the hydroxyls. Hence stops the PET process and fluorescence enhances. This also explains the different nature of cyclic voltammograms of **L** in presence of Cr^{3+} and Ce^{4+} , since Cr^{4+} affects the conjugation system of **L**, its presence greatly influences the cyclic voltammogram of **L** while Cr^{3+} does not affect the conjugation system of **L** and that is why the cyclic voltammogram is similar to that of **L**.

AND Logic Gate

The response of Ce^{4+} and AA towards **L** with respect to fluorescence forms the basis of AND Logic Gate truth table (Table 1, Scheme 3). Here the fluorescence generation has been assigned as 1 while no fluorescence enhancement has been assigned as 0.

Declarations

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Conflict of interest There are no conflict to declare.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Author contribution: BB has done all the experiments works; DKD has done analysis of the results and written the paper.

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Tables

Table 1

INOUPS		OUTPUTS
Ce ⁴⁺	AA	AND
0	0	0
1	0	0
0	1	0
1	1	1

Figures

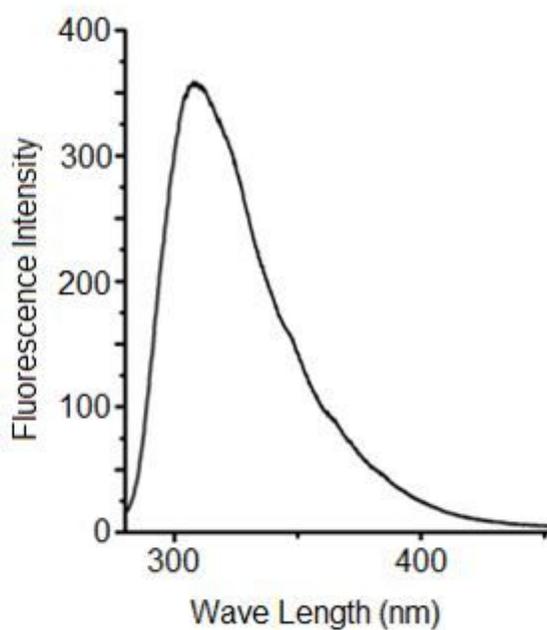


Figure 1

Fluorescence spectrum of L recorded in 1:1 CH₃OH:H₂O (v/v). (λ_{ex} 270nm, λ_{max} 308 nm)

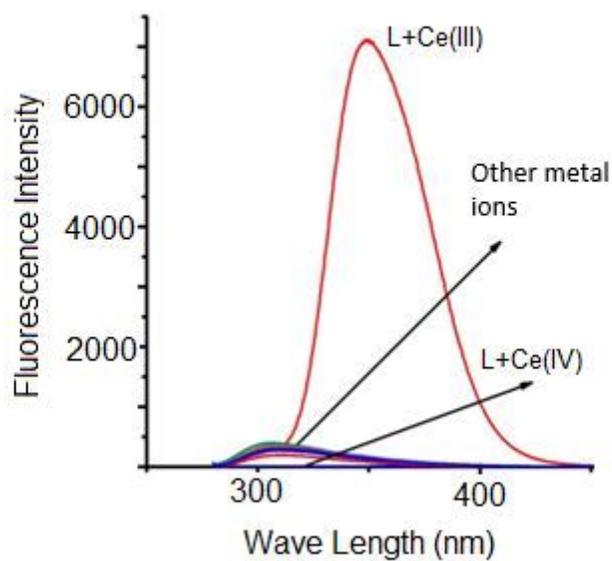


Figure 2

Fluorescence spectra of L in 1:1 (v/v) CH₃OH:H₂O on addition of 100 μ L of different metal solutions, λ_{ex} 270nm.

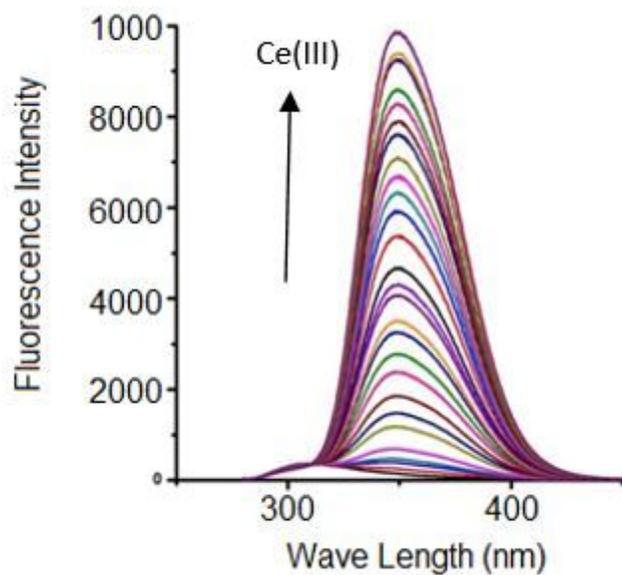


Figure 3

Fluorescence spectra of L in 1:1 CH₃OH:H₂O (v/v) at different added concentration of Ce³⁺, λ_{ex} 270nm, λ_{max} 348.6nm.

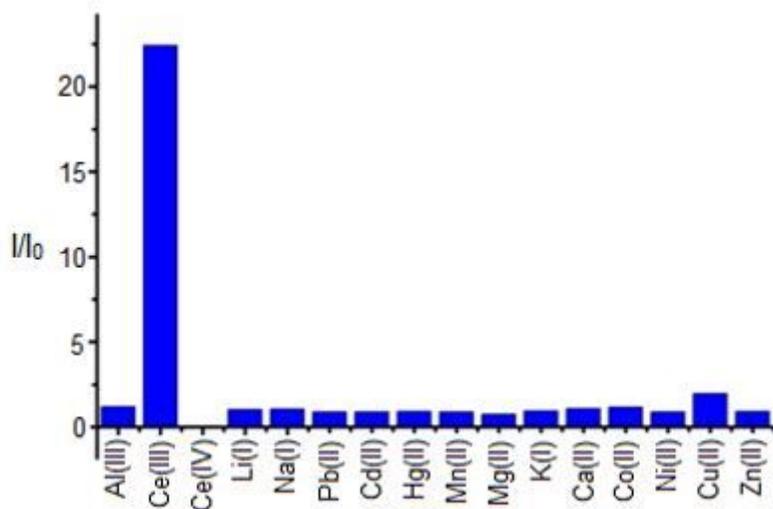


Figure 4

Bar diagram representing I/I₀ values for L in presence of different metal ions

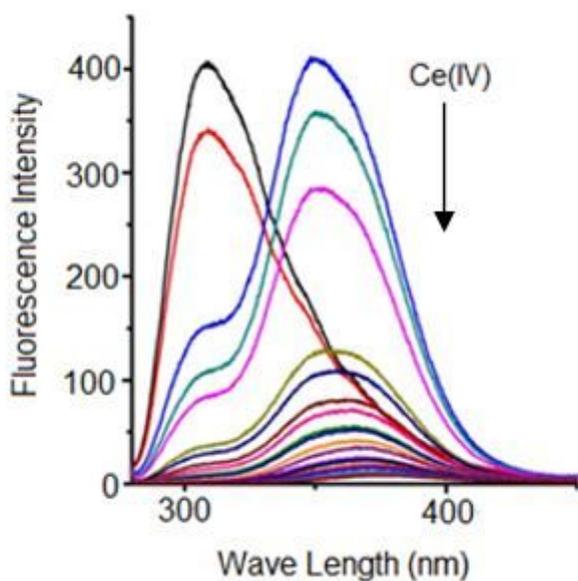


Figure 5

Fluorescence spectra of L in 1:1 (v/v) CH₃OH:H₂O at different added concentration of Ce⁴⁺, λ_{ex} 270 nm.

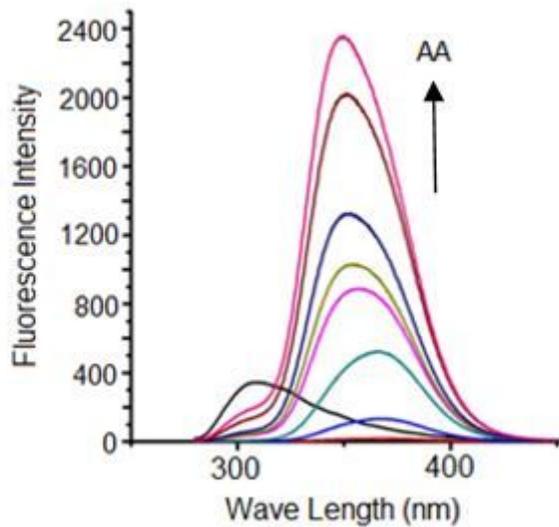


Figure 6

Fluorescence spectra of L with Ce⁴⁺ in 1:1 (v/v) CH₃OH:H₂O at different added concentration of ascorbic acid, λ_{ex} 270 nm, λ_{max} 350 nm.

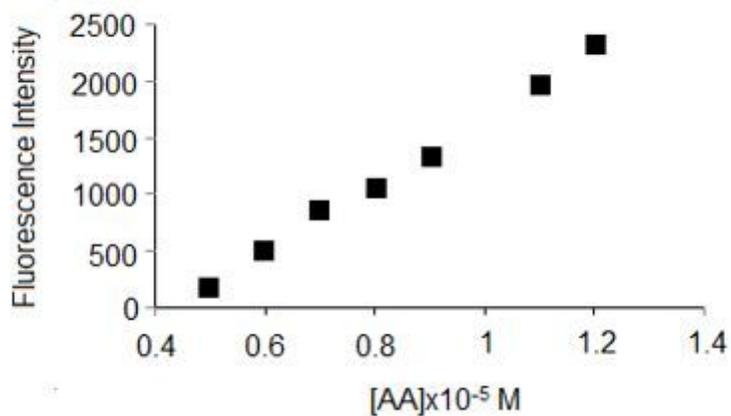


Figure 7

Fluorescence intensity of L:Ce⁴⁺ as a function of AA in 1:1 (v/v) CH₃OH:H₂O, λ_{ex} 270 nm, λ_{max} 350 nm.

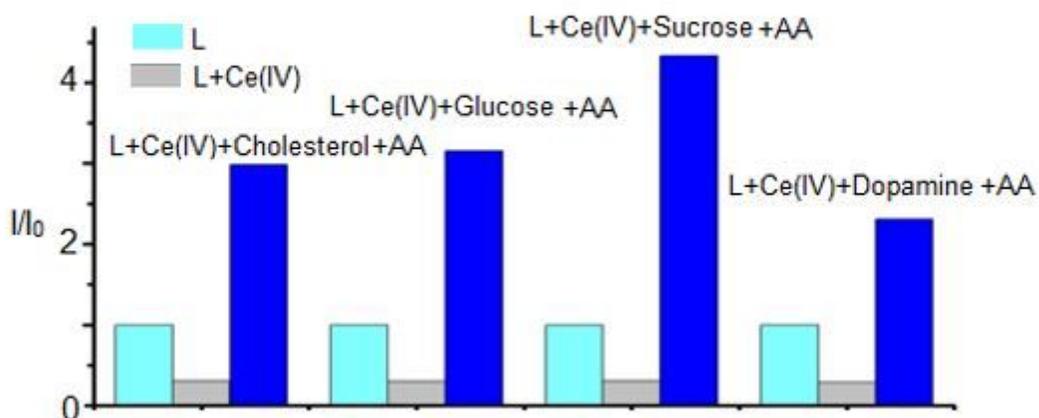


Figure 8

Plot of I/I_0 response of L (Green bars), L with Ce(IV) (Grey bars) and L, Ce(IV) + AA + other species (Blue bars) in 1:1 CH₃OH:H₂O (v/v)

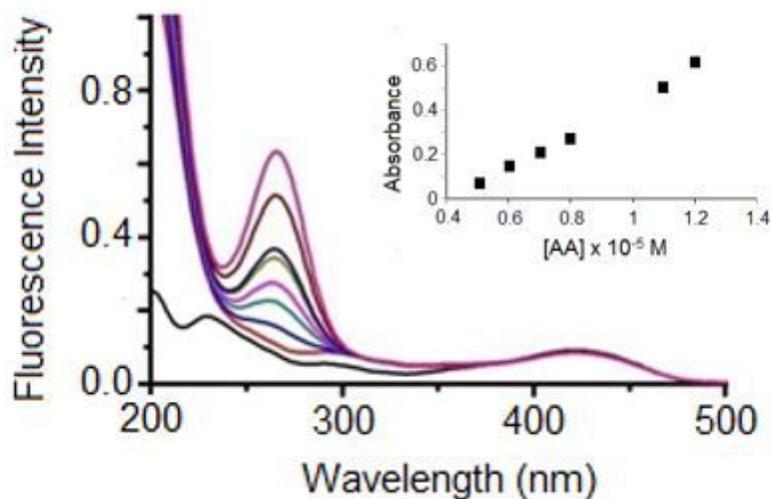


Figure 9

Change in the UV/Visible spectrum of L in 1:1 (v/v) CH₃OH:H₂O in presence of Ce⁴⁺ and different added concentration of Ascorbic acid.

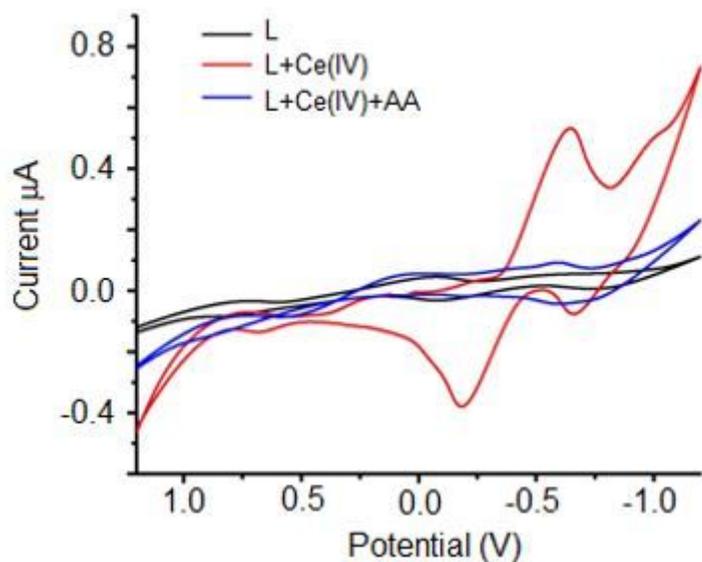


Figure 10

Cyclic voltammogram response of L in 1:1 (v/v) CH₃OH:H₂O WE-Pt electrode, RE-Ag/AgCl, Scan rate 0.1V/s.

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