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Bovine serum albumin loaded Boron doped carbon dots as a sensing probe for the detection of Pb (II) ion in water samples

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Abstract

Carbon dots have excellent potential to be utilized for biochemical sensing and environmental testing due to its unique optical properties, excellent biocompatibility and are readily surface functionalized. Herein, the boron doped carbon dots synthesized by microwave treatment process using one pot approach. The maximum excitation wavelength is 345 nm and emission is found at 444 nm. It shows excellent blue fluorescence when excited under uv light. Potential of carbon dots as optical sensor has been tested using different cations, but it is found that Pb(II) ion shows maximum and selective quenching. Furthermore stern-volmer relationship is established, by using fluorescence quenching spectra. The stern-volmer constant and limit of detection is found to be $2.39 \times 10^3 M^{-1}$ and 10ppb respectively. This study will be helpful in development of new fluorescent nano-biosensors.

Key words :- Carbon dots, fluorescence, stern-volmer, nano-biosensor.

1. Introduction

The issue of environmental pollution and its effects on human health have become more and more consequential. Fluorescent sensors have the benefits of high sensitivity and rapid analysis for environmental sample [1]. Fluorescent sensors such as carbon dots are less toxic and biocompatible as compared to semiconductor quantum dots. Synthesis and implementation of fluorescent sensors are easy and cost effective [2]. CDs are a member of carbon nanomaterial family with diameter below 10 nm have excellent fluorescent performance, dispersity and biocompatibility [3,4]. The synthesis

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methods of CDs have two major approaches one is bottom-up, another one is top-down. In top-down approaches size of large molecules as graphite is reduced until the products become fluorescent nanoparticles. While in the bottom-up approaches any which is carbogenic in nature can produce CDs under suitable conditions[5]. Every time the synthetic process, starting materials affect the properties of synthesized nanomaterials such as size, crystallinity, heteroatom content such as oxygen, nitrogen, sulphur or boron. It also affects the optical properties of CDs such as fluorescence emission and biocompatibility with used solvents[6-9]. Specially, with the significance of excellent dispersibility in water and tunable fluorescence, CDs are selected as novel fluorescent probes and optical sensors[10,11]. Carbon nanomaterials were accidentally discovered by Xu and co-workers[12] during the purification of carbon nanotubes, from this invention research activity in the following field have been continuously growing. Now a days these nanoparticles are well known as Carbon dots (CDs) [13]. The synthesis of carbon dots can be divided into three major steps; carbonization, passivation, and surface functionalisation[14] in carbonisation precursor is converted into basic carbon element by pyrolysis, dehydration or destructive distillation. Chemical treatment of carbon dots known as passivation. In functionalisation step specific groups like $-\text{COOH}$ and $-\text{NH}_2$ are embedded on the surface of CDs using specific reagents[15]. In the present work Pb(II) ion have been detected successfully upto 0.5 ppm, using BSA loaded boron doped carbon dots as fluorescent sensing probes and Stern-volmer relationship have also been established.

2. Experimental

2.1 Reagents

The entire chemical used in this work were of analytical grade. Ultrapure water (prepared from Merck Millipore system) was employed as a solvent throughout the work. Bovine serum albumin obtained from Loba chemie pvt.ltd, and boric acid (H_3BO_3), citric acid ($\text{C}_6\text{H}_8\text{O}_7$) and urea (NH_2CONH_2) were procured from sigma Aldrich pvt.ltd, received without further purification.

2.2 Instrumentation

Steady state fluorescence measurements were performed using fluorescence spectrophotometer (CARY Eclipse, Varian) Fluorescence spectra different concentrations of BCDs were recorded in the 400-600 nm range. The appropriate excitation wavelength was 268nm. Scan rate of instrument was 500 nm min^{-1} . The excitation slit, and emission slit of the instrument were taken as 5.0 and 5.0 nm respectively. For the study of morphology of CDs, transmission electron microscope (TEM) measurements were performed on a JEOL, JEM-2100F, operated at accelerating voltage 200 kV. For FTIR spectra diffuse reflectance method was employed on a Carry Eclipse Fourier Transform infrared spectroscopy by using liquid sample ATR. In the range of $1400-1700 \text{ cm}^{-1}$ FTIR spectra of Bovine serum albumin alone and with BCDs were taken. UV-visible measurements were taken from 200 nm to 600 nm wavelength. Wavelength range of 200-400 nm in 0.1 M CPB (pH 7.4) at 25°C was fixed UV-Vis absorption spectra of BCDs and BSA-BCDs system also. Reagent Blank buffer are used as reference. A pH metre (Mettler Toledo) A pH metre was used for pH measurement of samples. Water bath was also used for maintenance of temperature during the study.

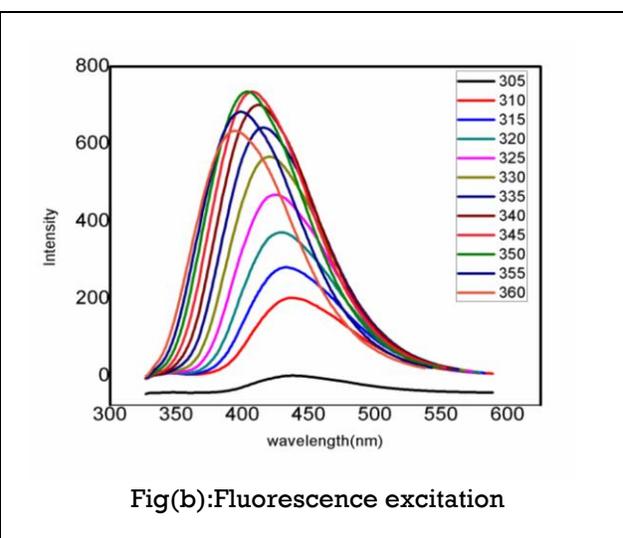
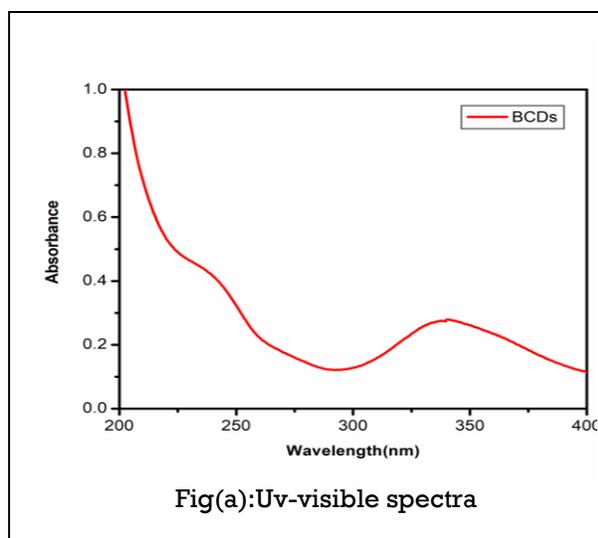


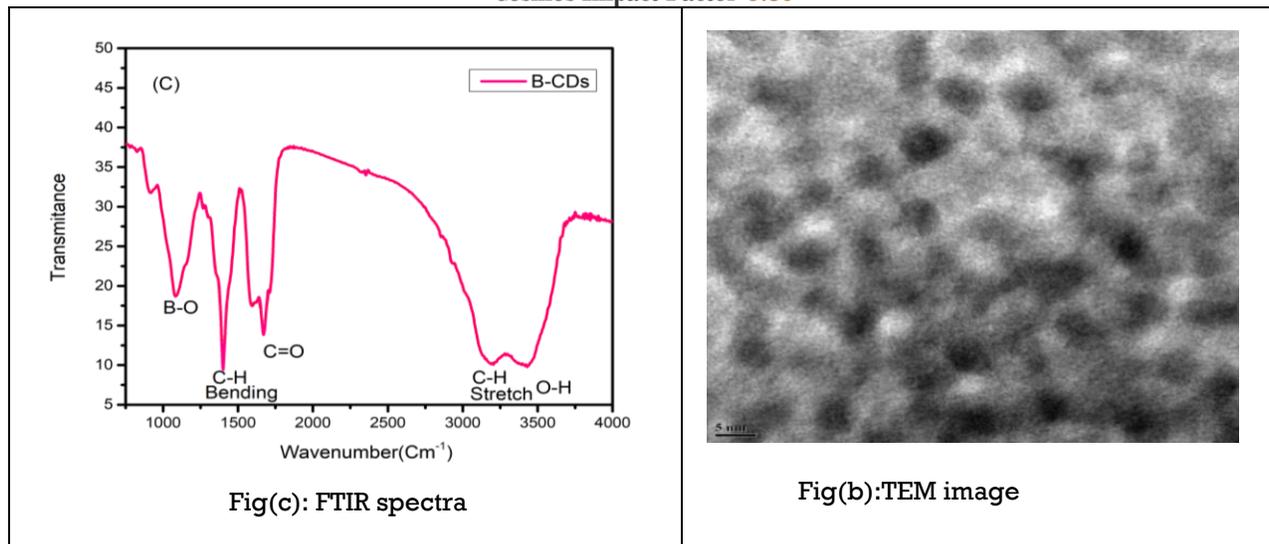
2.3. Synthesis of Boron doped carbon dots

B-CDs were synthesized by the previously reported method [16]. Initially 1g urea, 1g citric acid and 1g boric acid were dissolved in 20 ml of ultrapure water. This mixture was treated under microwave oven at 700 W for 4 minutes. The obtained material was olive-green solid, which was primary indication of preparation of BCDs. The resulting solid were re-dissolved in 20 ml distilled and further purified by dialysis for 24 hours to eliminate the impurities. Then obtained B-CDs solutions were kept at 4°C for further analysis.

2.4. Characterisation of BCDs

Bands for BCDs are found in between 200 nm to 360 nm which is an indication of carbon nanomaterials. UV-Vis peaks at 282nm indicates the $\pi-\pi^*$ transition of sp^2 aromatic system. BCDs shows fluorescence Band at 444nm. Diluted solution of BCDs is light-green in colour and show blue fluorescence upon excitation with UV (365nm). Fluorescence of BCDs depends upon excitation wavelength. On excitation from 305nm to 360 nm fluorescence intensity regularly increases on increasing wavelength and shows persistent red shift and maximum intensity observed at 345 nm and emission at 444 nm excitation dependent fluorescence is a result of various surface states of carbon-based nanomaterials. The stability of BCDs was also analysed by taking fluorescence and uv-visible spectral measurement at wide range of pH (2.0 to 10.0). BCDs shows high photo stability at room temperature in wide range of pH. The functional groups which are present on the surface of BCDs can be identified using FTIR spectrum. The absorption bands at 3450 cm^{-1} and 3100 cm^{-1} are related to O-H and C-H stretch respectively. Bands at 1450 and 1680 cm^{-1} are of C-H bending and C=O respectively. The absorption band at 1031 cm^{-1} indicates B-O stretching. These observations tell us that BCDs were functionalized by -COOH and -OH groups. The availability of oxygen containing Functional groups are responsible for water solubility and interaction with metal ions and bio molecules.





2.5. Capping of BSA on the surface of BCDs

A equimolar mixture of BSA and BCDs were kept for continuous stirring for 24 hours. Surface modification of BCDs using BSA can be identified by significant spectral changes in its fluorescence properties as shown in figure below it is well known that BSA is also fluorescent due to two tryptophan (Trp) residues located in the surface of protein; domain 1 (Trp-134) and in the hydrophobic pocket in domain 2 (Trp-214)[17]. Peak at 444nm is due to BCDs and at around is due to BSA modified BCDs.

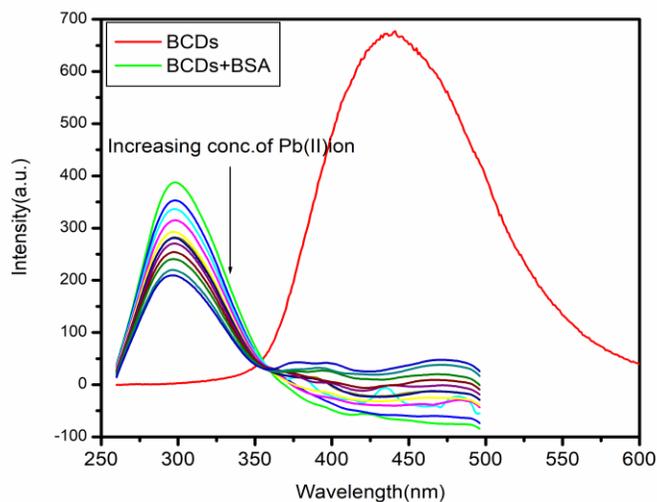
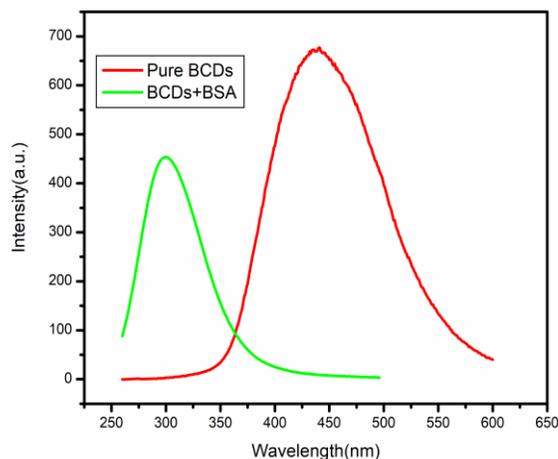


Fig (e): Capping of BCDs with BSA



Fig(f): Detection of Pb(II) ion

3. Results and discussion

3.1. Detection of Pb(II) ion

The binding between Pb(II) ion and –COOH moieties of BSA modified BCDs destroyed the stability luminescent properties which results in fluorescence quenching. The d-orbital's of Pb(II) received electrons in the excited state of BSA modified BCDs via nonradiative electron transfer. The fluorescence of BCDs was Quenched linearly to Pb(II) with a detection limit 10 ppb.

3.2. Fluorescence sensing

Fluorescent sensing Under different conditions of temperature and same condition of pH and ionic strength fluorescence quenching is due to the ground state complex formation in between quencher Pb(II)ion and BCDs. When the fluorophore and quencher molecule come into contact during lifetime of excited state then it referred to as Dynamic quenching, whereas in static quenching there is fluorescence-quencher complex formation. Fluorescence quenching for BCDs-Pb(II) mechanism was studied using Stern-Volmer equation[18]

$$\frac{F}{F_0} = 1 + K_{SV} [Q] \quad \dots\dots\dots (1)$$

Where F and F₀ are the fluorescence intensities of BSA modified BCDs in the presence and absence of Pb(II) ion respectively. Stern-Volmer quenching constant is K_{SV} and [Q] is the concentration of quencher i.e. Pb(II) ion. The value of K_{SV} can be calculated using linear fit graph between F₀/F and [Q]. Results obtains reveals that there is dynamic quenching mechanism.



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