Synthesis of bovine serum albumin capped boron-doped carbon dots for sensitive and selective detection of Pb(II) ion

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ABSTRACT

Carbon dots have tremendous potential to be used for biochemical sensing and environmental testing due to its superior optical properties and excellent biocompatibility. The surface of carbon dots can be easily functionalized. In the present study boron doped carbon dots have been synthesized using one pot approach by microwave treatment method. The surface of boron doped carbon dots is capped with bovine serum albumin. The maximum fluorescence emission observed at 444 nm when excited upon 345 nm of wavelength. In the normal light, it is light green in colour but when exposed in long wavelength UV light it exhibited blue fluorescence. The carbon dots have an irregular shape with a diameter below 5 nm. The applicability of synthesized carbon dots as the fluorescent sensor has been checked using different metal ions. It is observed that Pb(II) ion shows appreciable and selective quenching. Linear relationship is exist between the decrease in fluorescence intensity and the concentrations of Pb(II) ion in the range from 1 ppb to 10 ppb concentration. Limit of detection is found to be 0.08 ppb. This study will be helpful in the development of new fluorescent nano-biosensors.

1. Introduction

The environmental pollution has adverse effects on human health and is increasing day by day due to the increased level of pollutants. Fluorescent sensor has advantages of selectivity, sensitivity and fast analysis of environmental samples [1]. Carbon dots as a fluorescent sensor has less toxic and biocompatible when compared to semiconductor quantum dots. Synthesis and application of fluorescent sensor are cheap and very convenient [2]. Carbon dots (CDs) with a size below 10nm shows outstanding fluorescence, biocompatibility and dispersibility in water is the part of carbon nanomaterial family [3, 4]. The synthesis methods of CDs can be classified such as top down and bottom up [5]. Properties of prepared CDs i.e. size, crystallinity, heteroatom content such as oxygen, nitrogen, sulphur and boron depends upon starting material and synthesis processes. It also alters the optical properties of CDs such as fluorescence emission and biocompatibility when used with different solvents [6, 7, 8, 9]. CDs show excellent dispersability in water and also tunable nature of fluorescence makes them novel and optical sensing probes [10, 11]. Surface modification can introduce variety of functional groups such as –COOH, –OH, –NH2 on the surface of carbon dots, for this purpose various polymers and organic molecules have been used. Surface passivation may alter the wavelength of emission and increases the selectivity towards particular ions or a molecule etc. Hence this characteristic nature of CDs enables the applicability of carbon dots in detection or sensing. The functional groups present on the surface of carbon dots are responsible for binding with metal ions [12]. Lead is a well-known toxic heavy metal, it can be accumulated in kidneys, brain and nervous system, which may cause damage and dysfunction of central nervous system in human beings [13]. Different carbon dot based nano-sensors have been developed for detection of Pb(II) ion (Table 1). From Table 1, although these methods have appreciable detection limit (LOD), but these methods often require complicated synthesis, modifying process, expensive reagents and instruments.

In this work boron doped carbon dots (BCDs) were synthesized by one-pot approach using boric acid, citric acid and urea as precursor by microwave treatment process. BCDs were capped with bovine serum albumin (BSA) and implemented as a fluorescent sensor for determination of Pb(II) ion. Fluorescence of BSA capped BCDs is restored ethylene diamine tetra acetato(EDTA) metal chelator. The principle of fluorescence quenching and recovery is shown in Scheme 1. Furthermore, the quenching mechanism is also explored. Here capping of BCDs with BSA provides novel probe for detection of Pb(II) ion.

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Table 1. Comparison between BSA capped BCDs sensor with other sensors.

| Substrate                  | Preparation method      | Metal ion detected | Detection range   | LOD   | References |
|---------------------------|-------------------------|--------------------|-------------------|-------|------------|
| Microalgae biochar        | Oxidation and autoclave | Pb(II)             | 0.01 μM-2mM       | 2.08ppb | [21]       |
| Chocolate                 | Electric heat           | Pb(II)             | 0.03μM-1.67μM     | 2.4ppb | [20]       |
| Orange peels              | Ultrasonication         | Pb(II), Hg(II), Cu(II), Ni(II), Ag(II), Sn(II) | 0.4 mM | 251 ppb | [22]       |
| Graphene; Au-NCs          | Chemical oxidation      | Pb(II)             | 50-1000 nM        | 2.08ppb | [23]       |
| Cysteamine and 11-mercaptoundecanoic acid | Chemical method | Pb(II) | 0.22-4.51 ppm | 30ppv | [24] |
| Au-NPs-DNA Zyme           | Chemical method         | Pb(II)             | 0.4-2 μM          | 83.2ppb | [25]       |
| Citric acid, Boric acid, Urea | Microwave treatment    | Pb(II)             | 1-10 ppb          | 0.08 ppb | This work |

2. Experimental

2.1. Reagents

All chemicals used in present work were of analytical grade without any further modification. Ultrapure water (obtained from Merck Millipore system, Germany) was used as a solvent throughout the experiment. Bovine serum albumin (>99%) purchased from Loba Chemie Pvt. Ltd., Mumbai, and boric acid(98%), citric acid(99%), urea(97%) and standard solutions of different metal ions were brought from Merck specialities Pvt. Ltd., Mumbai. Disodium EDTA (99%) and Citrate phosphate buffer were purchased from Tokyo Chemical Industry (India) Pvt. Ltd.

2.2. Instrumentation

Fluorescence spectra measurements were performed using Varian Cary Eclipse fluorescence spectrophotometer (Agilent technologies, Australia). The fluorescence spectrum was measured in the range of 200–600nm. The excitation wavelength of instrument was 270 nm. The ultraviolet-visible (UV-Vis) spectra were performed on Cary-50 UV-Vis spectrophotometer (Agilent technologies, Australia). High resolution Transmission electron microscope (TEM) images were obtained using JEOL, JEM-2100F (JAPAN), with accelerating voltage 200 kV. FTIR spectra were obtained by Thermo fisher DRS-FTIR instrument (U.S.). Liquid sample attenuator was applied for the analysis. Operation range of FTIR instrument was 1000–4000cm⁻¹. The pH of samples was measured in pH meter (Mettler Toledo, USA). The temperature was maintained using water bath.

2.3. Synthesis of BCDs

BCDs were synthesized by dissolving 1g urea, 1g citric acid, and 1g boric acid in 20 ml of ultrapure water. This mixture was kept in microwave oven (LG, MC2886BFUM, India) for 4 min at 700 W. The resulting material was olive-green solid. The obtained solid was dissolved in 20 ml of ultrapure water and this solution was purified by dialysis membrane (0.5kDa cut off) for 24 h to discard the impurities. Then purified solution of BCDs was kept at 277K for further use [14]. BCDs synthesized as aqueous dispersion and can be solidified by evaporation of water.

2.4. Characterization of BCDs

Carbon based nanomaterial shows absorption bands between 200-400 nm, UV-Visible spectrum absorption band at around 240nm due to π-π* transition of sp² aromatic carbon and absorption band at around 340nm is due n-π* transition as shown in Figure 1a. Fluorescence Band at 444nm was observed for BCDs. Colour of dilute solution of BCDs is light green but exhibited excellent blue fluorescence when excited upon long wavelength UV-light (365nm). Fluorescence of BCDs depends upon excitation wavelength. BCDs show excitation dependent fluorescence behaviour when excited upon different wavelength of light. Solution of BCDs when excited from 305 to 360 nm it show maximum emission at 345 nm of excitation wavelength [15]. As shown in Figure 1b.

With the help of FTIR spectrum functional groups which are present on the surface of BCDs can be examined. The absorption band at 3544 cm⁻¹ and 3289cm⁻¹ are due to O–H stretching. C–H stretching observed at 2875 cm⁻¹. Absorption band at1730 cm⁻¹ is related to C=O stretching respectively. B–O stretching band observed at 1072 cm⁻¹. Absorption band at 1242 cm⁻¹ is due to C–O stretching, absorption band C=C stretching is observed at 1454 cm⁻¹ and absorption band for C=C bending observed at 945 cm⁻¹. Change in transmittance on capping of BCDs with BSA was observed. New band at 1104 cm⁻¹ is due to C–O stretching vibration of C=O bonds, bands at around 1348cm⁻¹ are due to C–N stretching. After capping the BSA on the surface of BCDs, BCDs surface enriched with –OH, –COOH and –NH₂ functional groups. As shown in Figure 1c.
The particle size and morphology of synthesized BCDs can be analyzed by high resolution transmission electron microscopy (HRTEM). HRTEM analysis shows that spherical shape and around 5 nm sizes of quantum dots. As shown in Figure 1d.

2.5. Surface modification of BCDs using BSA

For the surface modification of BCDs by BSA, 10⁻⁶ M concentrations and volume ratio 1:1 of both were allowed for continuously 24 h stirring. Successful capping of BSA on the surface of BCDs was indicated by spectral changes in its fluorescence properties. BSA is itself fluorescent molecule because of two tryptophan (Trp) residues situated on the protein surface; domain 1(Trp-134) and in the hydrophobic domain 2 (Trp-214) [16]. Fluorescence bands at 444 nm and around 300 nm are due to pure BCDs and BSA modified BCDs respectively, as shown in Figure 2a.

3. Results and discussion

3.1. Fluorescence sensing of Pb(II) ion by nano-sensing probe

Functional groups such as –COOH, –OH and –NH₂ are present on the surface of BSA capped BCDs are responsible for binding with Pb(II) ion. Mainly oxygen containing functional groups have more affinity towards metal ion. Pb(II) ion has more affinity towards oxygen containing functional groups than other metal ions because in the case of lead poisoning in the body following salt CaNa₂EDTA has been used to remove Pb (II) ion from the body, since EDTA ⁴⁻ is a hexadentate ligand containing four donor oxygen and two nitrogen donor site and BSA capped BCDs is enriched with –NH₂ and –COOH functional which shows efficient binding with Pb (II) ion. Binding between the functional groups and metal ion destroys the fluorescence characteristics of BSA capped BCDs results in continuous fluorescence quenching. The Capability of nano-sensing probe to quantitatively detection of Pb(II) ion was also investigated.

3.2. Procedure for detection of Pb(II) ion

Fluorescence intensities of BSA capped BCDs (0.5 mg mL⁻¹) solutions with increasing concentrations of Pb(II) ions (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ppb) are shown in Figure 2b. After interpretation of results obtained from fluorescence quenching experiments, It is clearly observed that fluorescence intensity of BSA capped BCDs gradually decreases with increasing concentration of Pb(II) ions. It is due the non-fluorescent complex formation between Pb(II) ion and BSA capped BCDs, as shown in Scheme 1. Limit of detection of Pb (II) ion can be determined using following Eq. (1)

\[
\text{LOD} = 3\sigma / m
\]
where \( \sigma \) is standard deviation and \( m \) is the slope.

Fluorescence quenching usually achieved either dynamic or static quenching. Stern-Volmer [17] reported dynamic quenching represented by Eq. (2) and static quenching represented by Eq. (3)

\[
\frac{F}{F_0} = \frac{\tau_0}{\tau} = 1 + K_q \tau_0 [Q]
\]

\[
\frac{F}{F_0} = 1 + K_{SV} [Q]
\]

where \( K_q \) is the rate constant of dynamic quenching; \( \tau_0 \) is the lifetime of fluorophore in the absence of quenchers; \( \tau \) is the lifetime in the presence of quenchers. \( K_{SV} \) is the static quenching constant and \( [Q] \) is the concentration of quencher in the solution. The value of \( K_{SV} \) can be calculated using a linear fit graph between \( F_0/F \) and \( [Q] \) from using the Stern-Volmer plot.

Drawn a plot between \( F_0/F \) and concentration of Pb (II) in the range of 0–10 ppb and fitted linearly as shown in Figure 2c. We can predict that the quenching mechanism of our work is either one of static or dynamic quenching modes. To further confirmation of the quenching type, we investigated the recovery of fluorescence intensity. When Pb(II) ion is added to the solution of BSA capped BCDs, the fluorescence intensity is quenched with respect to non Pb(II) ion solution. On addition of metal chelate CaNa₂EDTA (10⁻³ M) to Pb(II) ion mixture solution the fluorescence intensity of the mixture solution is recovered. Because at that time Pb(II) ion is coordinated with EDTA⁻⁴ in the form of \([\text{Pb(EDTA)}]⁻²\), instead of BSA capped BCDs shown in Figure 3a. These observations show that mechanism of fluorescence quenching is static.

3.3. Optimization of BSA capped BCDs solution as fluorescent sensing probe for detection of Pb(II) ion

For the detection of Pb(II) ion by the BSA capped BCDs solution were optimized under suitable experimental parameters. Dependency of quenching efficiency of BSA capped BCDs solution was optimized by two parameters (pH and concentration of analyte Pb (II) ion). We have chosen pH because it plays a vital role in the complex formation between BSA capped BCDs and Pb(II) ion. It is evident that more the quenching efficiency more will be the complexation between the sensor and metal ion. Quenching efficiency is tested in a wide range of pH (2.0–10.0), it is found that around 7.4 pH gave highest quenching efficiency i.e. more complex formation [18] as shown in Figure 2d. Fluorescence intensity is investigated with respect to different time intervals, it is found that fluorescence intensity remains unchanged with time i.e. quantum dot is very stable as shown in Figure 3b. Fluorescence intensity is also
3.4. Selectivity for Pb (II) ion determination

A series of different metal ions (K⁺, Na⁺, As⁺³, Co²⁺, Ag⁺, Cr⁺³, Hg⁺², Mg⁺², Cu⁺², Ni⁺², Cu⁺², Fe⁺³, Ca⁺²) are tested separately in the solution of BSA capped BCDs to evaluate the selectivity of nano-sensor towards Pb (II) ion. Firstly the same concentration (1–10 ppb) of Pb(II) and other potentially interfering ions were used. As shown in Figure 3d. The highest and significant quenching is observed in the presence of Pb(II) ion. In further experiments of selectivity investigation even 50 times more concentration of other interfering ions not shown any significant fluorescence quenching, based on these observations we can conclude that BSA capped BCDs is a very selective nano-biosensor for the detection of Pb(II) ion.

4. Conclusions

In the present work BSA capped BCDs shows very good fluorescence characteristics under UV radiation and it shows excellent stability under different ranges of pH, however fluorescence intensity depends on pH, around 7–8 pH it shows the maximum intensity of fluorescence, since BCDs is capped with BSA which is a biomolecule and biomolecules are stable at physiological pH i.e. 7-8, so fluorescence intensity found maximum at 7.4 pH. Fluorescence of capped BCDs has been effectively quenched by Pb(II) ion. Based on this result BSA capped BCDs were employed for sensitive detection of Pb(II) ion as a nano-sensing probe which is novel and economic. Findings of this work is also compared with previously reported studies (Table 1) it is found that method is sensitive than other ones.

Declarations

Author contribution statement

Fahmida Khan: Conceived and designed the experiments. Vinayak Sahu: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.
Additional information

No additional information is available for this paper.

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