Heliyon 6 (2020) e03957

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

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

Vinayak Sahu, Fahmida Khan *

Department of Chemistry, National Institute of Technology, Raipur, Chhattisgarh, 492010, India

ARTICLE INFO

Keywords:
Materials chemistry
Nanotechnology
Carbon dots
Fluorescence
Nano-bio sensor
Metal ions

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 dispensability 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,-NH_2 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 effectively quenched by Pb(II) ion as compared to other metal ions. Furthermore, the fluorescence of quenched Pb(II)-BSA capped BCDs is restored ethylene diamine tetra acetato($EDTA^{-4}$) 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.

* Corresponding author.
E-mail address: fkhan.chy@nitrr.ac.in (F. Khan).

https://doi.org/10.1016/j.heliyon.2020.e03957
Received 5 November 2019; Received in revised form 12 April 2020; Accepted 6 May 2020

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	[19]
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	251ppb	[21]
Graphene; Au–NCs	Chemical oxidation	Pb(II)	50–1000 nM	2.08ppb	[22]
cysteamine and 11-mercaptoundecanoic acid	Chemical method	Pb(II)	0.22–4.51 ppm	30ppb	[23]
Au-NPs-DNA Zyme	Chemical method	Pb(II)	0.4–2 μM	83.2ppb	[24]
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 and Calcium disodium edetate($CaNa_2$**EDTA**) 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.

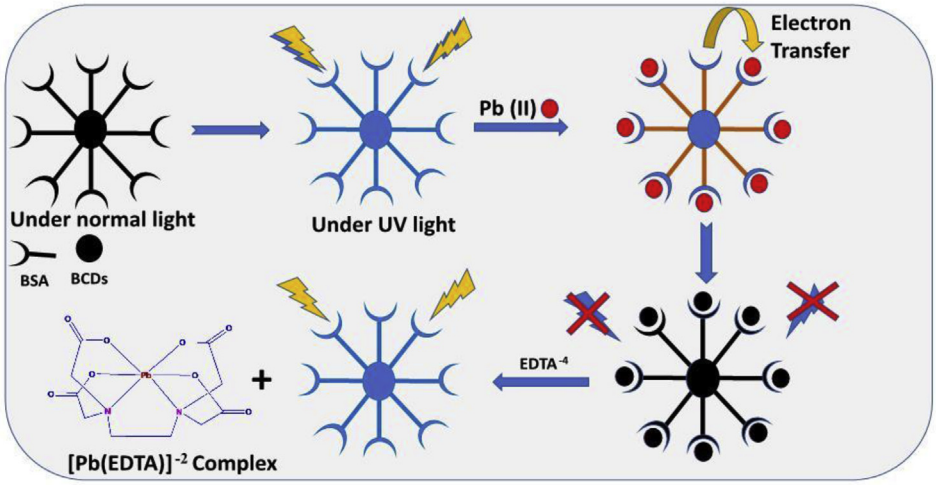
2.3. Synthesis of BCDs

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 sp^2aromatic 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 1104cm^{-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 $–NH_2$ functional groups. As shown in Figure 1c.



Scheme 1.

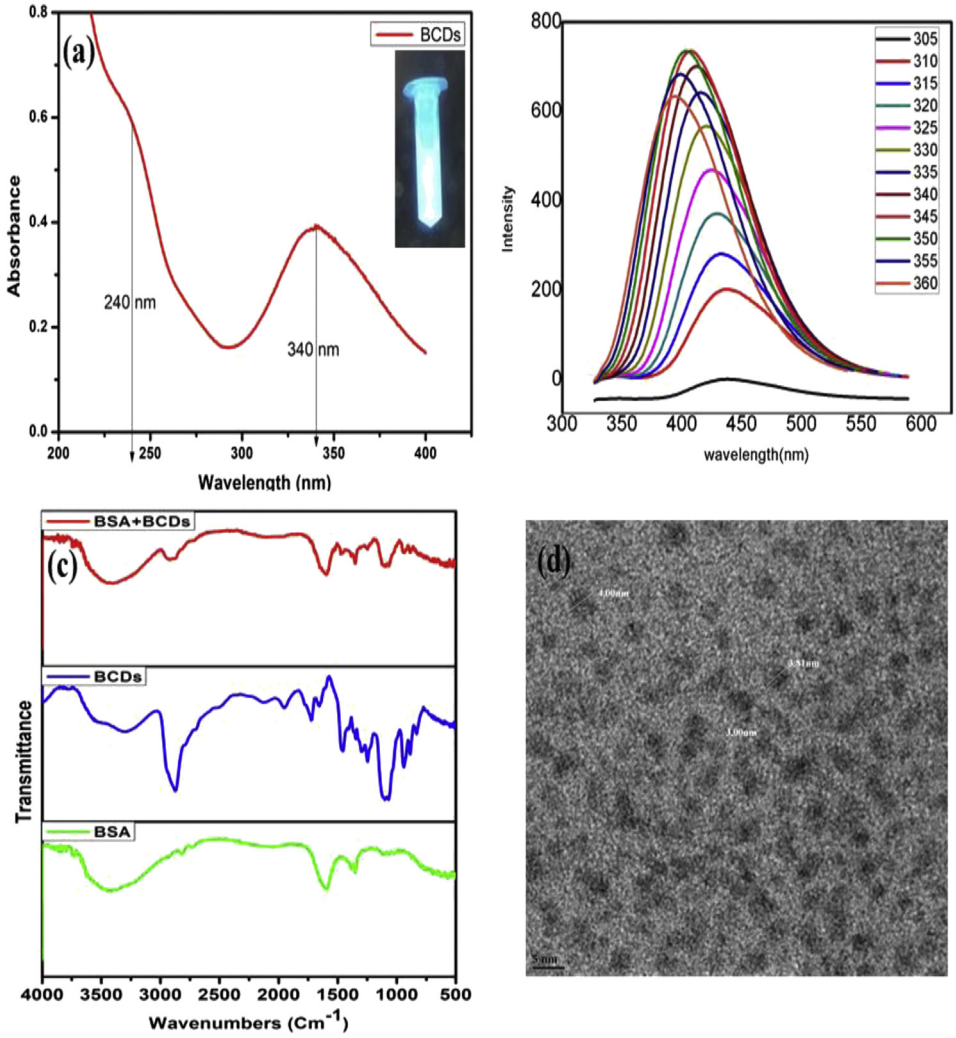


Figure 1. a. UV-visible spectra of BCDs in the range of 200 nm–400 nm wavelength, inset; photograph of BCDs under UV light b. Fluorescence spectra of BCDs at different excitation wavelengths. c. FTIR spectra of pure BSA, pure BCDs and BSA capped BCDs. d. HRTEM images of BCDs in water showing the different sizes of carbon dots.

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 $–NH_2$ 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 $CaNa_2EDTA$ 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 $–NH_2$ 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)

$$LOD = 3\sigma / m$$

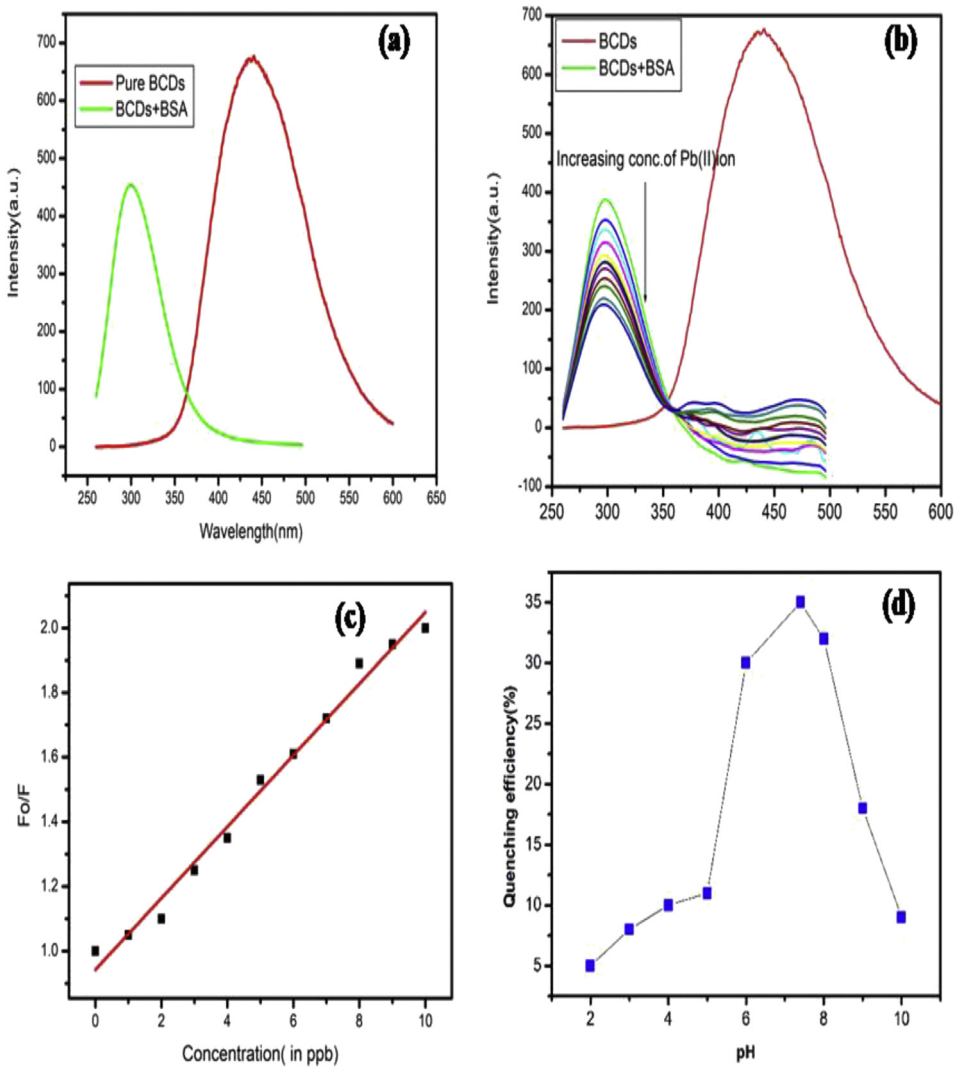


Figure 2. a. Fluorescence spectra of pure BCDs and with addition of BSA in solution. b. Fluorescence quenching spectra of BSA capped BCDs in the presence of 0–10ppb concentration of Pb(II) ion. c. Linear calibration graph for calculation of limit of detection (LOD) of Pb(II) ion. d. Effect of pH on the quenching efficiency of Pb(II) ion.

where **σ** 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)

$$\frac{F_0}{F} = \frac{\tau_0}{\tau} = 1 + K_q \tau_0 [Q] \quad (2)$$

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \quad (3)$$

where K_q is the rate constant of dynamic quenching; τ_0 is the lifetime of fluorophore in the absence of quenchers;τ is the life time 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_O/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 spectra intensity is quenched with respect to non Pb(II) ion solution. On addition of metal chelate $CaNa_2EDTA$ (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 $[Pb(EDTA)]^{-2}$, 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

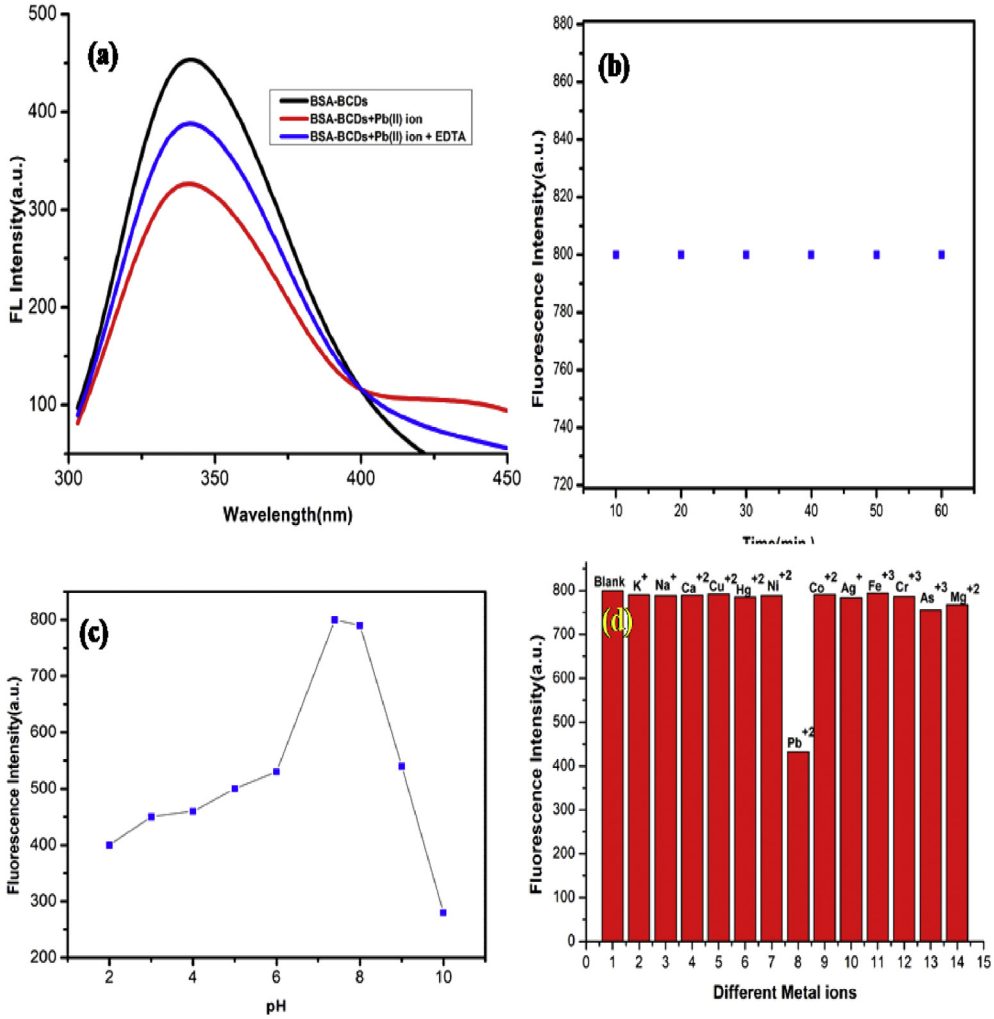


Figure 3. a. Fluorescence spectra in the favour of static quenching mechanism. b. Intensity of fluorescence of BCDs as a function of time. c. Intensity of fluorescence of BCDs as a function of pH. d. Selectivity of BSA capped BCDs sensor for Pb(II) ion the presence of other metal ions (Interference of different metal ions).

investigated at different pH, at 7–8 pH the maximum intensity is observed and shown in Figure 3c.

3.4. Selectivity for Pb (II) ion determination

A series of different metal ions (K^{+}, Na^{+}, As^{+3}, Co^{+2}, 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.

Funding statement

Vinayak Sahu was supported by Council for Scientific and Industrial Research, Library Avenue Pusa New Delhi (09/1116(0003)/2017-EMR-I).

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors are thankful to Director of National Institute of Technology Raipur(C.G.) for providing necessary laboratory facilities.

References

[1] Depeng Kong, Fanyong Yan, Dechao Shi, Qianghua Ye, Ziyi Han, Li Chen, Lue Wang, Carbon dots: synthetic methods and applications as fluorescent probes for the detection of metal ions, inorganic anions and organic molecules, J. Iran. Chem. Soc. 12 (10) (2015) 1841–1857.

[2] Meiting Dong, Jingyi Xu, Shuxian Liu, Ying Zhou, Chaobiao Huang, Synthesis of highly luminescent mercaptosuccinic acid-coated CdSe nanocrystals under atmospheric conditions, Luminescence 29 (7) (2014) 818–823.

[3] Shenghong Yang, Xiaohan Sun, Zhaoyan Wang, Xiayan Wang, Guangsheng Guo, Qiaosheng Pu, Anomalous enhancement of fluorescence of carbon dots through lanthanum doping and potential application in intracellular imaging of ferric ion, Nano Res. 11 (3) (2018) 1369–1378.

[4] Lei Wang, Shou-Jun Zhu, Hai-Yu Wang, Song-Nan Qu, Yong-Lai Zhang, Jun-Hu Zhang, Qi-Dai Chen, et al., Common origin of green luminescence in carbon nanodots and graphene quantum dots, ACS Nano 8 (3) (2014) 2541–2547.

[5] Zhenggang Wang, Boshi Fu, Siwei Zou, Bo Duan, Chunyu Chang, Bai Yang, Xiang Zhou, Lina Zhang, Facile construction of carbon dots via acid catalytic hydrothermal method and their application for target imaging of cancer cells, Nano Res 9 (1) (2016) 214–223.

[6] Minghan Xu, Guili He, Zhaohui Li, Fengjiao He, Feng Gao, Yanjie Su, Liying Zhang, Zhi Yang, Yafei Zhang, A green heterogeneous synthesis of N-doped carbon dots and their photoluminescence applications in solid and aqueous states, Nanoscale 6 (17) (2014) 10307–10315.

[7] Gaber Hashem Gaber Ahmed, Rosana Badía Laíño, Josefa Angela García Calzón, Marta Elena Díaz García, Fluorescent carbon nanodots for sensitive and selective detection of tannic acid in wines, Talanta 132 (2015) 252–257.

[8] Katerina Hola, Athanasios B. Bourlinos, Ondrej Kozak, Karel Berka, Karolina M. Siskova, Marketa Havrdova, Jiri Tucek et al. "Photoluminescence effects of graphitic core size and surface functional groups in carbon dots: COO- induced red-shift emission, Carbon 70 (2014) 279–286.

[9] Yu Zou, Fanyong Yan, Tancheng Zheng, Dechao Shi, Fengzhan Sun, Ning Yang, Li Chen, Highly luminescent organosilane-functionalized carbon dots as a nanosensor for sensitive and selective detection of quercetin in aqueous solution, Talanta 135 (2015) 145–148.

[10] Bingyan Han, Ying Li, Xixi Hu, Yan Qin, Jingmei Jiang, Mingbo Yu, Tingting Peng, Gaohong He, Based visual detection of silver ions and l-cysteine with a dual-emissive nanosystem of carbon quantum dots and gold nanoclusters, Anal Method 10 (32) (2018) 3945–3950.

[11] Li Wang, H. Susan Zhou, Green synthesis of luminescent nitrogen-doped carbon dots from milk and its imaging application, Anal. Chem. 86 (18) (2014) 8902–8905.

[12] Konstantinos Dimos, Carbon quantum dots: surface passivation and functionalization, Curr. Org. Chem. 20 (6) (2016) 682–695.

[13] Yang An, Yuanyuan Liu, Zeyan Wang, Peng Wang, Zhaoke Zheng, Ying Dai, Xiaoyan Qin, Xiaoyang Zhang, Myung-Hwan Whangbo, Baibiao Huang, Stabilizing the titanium-based metal organic frameworks in water by metal cations with empty or partially-filled d orbitals, J. Colloid Interface Sci. 533 (2019) 9–12.

[14] Athanasios B. Bourlinos, Georgios Trivizas, Michael A. Karakassides, Maria Baikousi, Antonios Kouloumpis, Dimitrios Gournis, Aristides Bakandritsos, et al., Green and simple route toward boron doped carbon dots with significantly enhanced non-linear optical properties, Carbon 83 (2015) 173–179.

[15] Sandeep Kumar Vaishnav, Indrapal Karbhal, Manmohan L. Satnami, Kallol K. Ghosh, Spectroscopic studies on in vitro molecular interaction of highly fluorescent carbon dots with different serum albumins, J. Mol. Liq. 255 (2018) 279–287.

[16] Ya-Ping Sun, Bing Zhou, Yi Lin, Wei Wang, KA Shiral Fernando, Pankaj Pathak, Mohammed Jaouad Meziani, et al., Quantum-sized carbon dots for bright and colorful photoluminescence, J. Am. Chem. Soc. 128 (24) (2006) 7756–7757.

[17] Xuehua Li, Zhiwei Zhao, Chen Pan, Ionic liquid-assisted electrochemical exfoliation of carbon dots of different size for fluorescent imaging of bacteria by tuning the water fraction in electrolyte, Microchim. Acta 183 (9) (2016) 2525–2532.

[18] Zhong-Xia Wang, Xian-He Yu, Feng Li, Fen-Ying Kong, Wei-Xin Lv, Da-He Fan, Wei Wang, Preparation of boron-doped carbon dots for fluorometric determination of Pb (II), Cu (II) and pyrophosphate ions, Microchim. Acta 184 (12) (2017) 4775–4783.

[19] J. Plácido, S. Bustamante-López, K.E. Meissner, D.E. Kelly, S.L. Kelly, Microalgae biochar-derived carbon dots and their application in heavy metal sensing in aqueous systems, Sci. Total Environ. 656 (2019) 531–539.

[20] Yongli Liu, Qingxiang Zhou, Li Jing, Man Lei, Xiuyi Yan, Selective and sensitive chemosensor for lead ions using fluorescent carbon dots prepared from chocolate by one-step hydrothermal method, Sensor. Actuator. B Chem. 237 (2016) 597–604.

[21] Adden Nguong Leong Tiong, Nicholas Kwong Hui Wong, Jessica Fung Yee Fong, Xian Wen Tan, Sing Muk Ng, A sustainable alternative to synthesis optical sensing receptor for the detection of metal ions, Opt. Mater. 40 (2015) 132–138.

[22] Xiuli Fu, Tingting Lou, Zhaopeng Chen, Meng Lin, Weiwei Feng, Lingxin Chen, "Turn-on" fluorescence detection of lead ions based on accelerated leaching of gold nanoparticles on the surface of graphene, ACS Appl. Mater. Interfaces 4 (2) (2012) 1080–1086.

[23] Xin Wang, Xiangqun Guo, Ultrasensitive Pb 2+ detection based on fluorescence resonance energy transfer (FRET) between quantum dots and gold nanoparticles, Analyst 134 (7) (2009) 1348–1354.

[24] Juewen Liu, Yi Lu, Accelerated color change of gold nanoparticles assembled by DNAzymes for simple and fast colorimetric Pb2+ detection, J. Am. Chem. Soc. 126 (39) (2004) 12298–12305.