In-vitro molecular interaction of boron doped carbon dots with human serum albumin

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Abstract. Carbon dots have drawn prodigious attention in biomedical and biological fields by virtue of its unique optical properties, but their effect on structure and behaviour of essential proteins have rarely been explored. In this study boron doped carbon dots (BCDs) which was synthesized by microwave treatment of citric acid, boric acid and urea, with maximum emission wavelength of 444 nm when excited upon long wavelength ultra-violet light. Synthesized BCDs were characterized by UV-Visible, FTIR, HRTEM and Fluorescence spectroscopy. Interaction between BCDs and human serum albumin (HSA) is carried out in physiological conditions. Stoichiometry of BCDs-HSA was found to be 1:1 and various thermodynamic parameters have also been calculated. The negative value of ΔG suggested that interaction between HSA and BCDs was spontaneous in nature.

1. Introduction
A novel class of carbon nanomaterial have been widely used in biomedical and biological field due their superior properties such as tunable fluorescence, photostability, excellent water solubility, resistance towards photo-bleaching, low toxicity and excellent biocompatibility. Adsorption of protein on the surface of NPs may changes the protein structure and may alter its biological activities. HSA is the major and most important protein of the blood plasma which is used as carrier in the circulatory system. It is produced in the liver and distributed in the blood plasma as a non-glycosylated protein. The binding interaction of serum albumin gives idea about clinical, bio-chemical and pharmaceutical applications [1-5]. HSA consists of three helical domains, and contain 585 amino acids each domain composed of two subdomains. Xu et al. studied the interaction between semi-conductor quantum dots i.e. CdTe and HSA, from this study it is revealed that CdTe binds to HSA and alters the structure of HSA [6]. Interaction between serum albumin and carbon based nanomaterials i.e. CDs and GQDs has also been reported but interaction between hetero atom doped carbon dots and HSA have rarely been explored.

In the present study synthesized BCDs have been used for in-vitro molecular interaction between BCDs and HSA has been done using multi spectroscopic techniques and various thermodynamic parameters were calculated.
2. Experimental

2.1 Materials
Boric acid, citric acid, urea was purchased from Sigma Aldrich Chemical Pvt. Ltd. Mumbai. HSA about 99% pure was procured from TCI chemical Pvt. Ltd. Warfarin and Ibuprofen were obtained from Pax Drug Company. All the chemicals used in the experiment were of analytical reagent grade.

2.2 Preparation and purification of BCDs
BCDs was prepared according to reported method [7] with slight modification, obtained raw solution of BCDs was dialyzed for 24 hours against distilled water to eliminate impurities. Purified BCDs was stored at 277 K for further use.

2.3 Characterization
In the UV-Visible spectrum of BCDs bands at 240 nm for $\pi-\pi^*$ and 340 nm for $n-\pi^*$ electronic transition obtained respectively. Fluorescence spectra of BCDs shows band at 444 nm. Colour of diluted solution of BCDs was light yellow and when it is exposed to UV light, it gives blue fluorescence as shown in inset of figure 1(a). When diluted solution of BCDs excited upon various excitation wavelengths it shows maximum fluorescence intensity at 444 nm when excitation wavelength 345 nm was applied. Figure 1(b). FTIR spectra confirm the presence of $-\text{COOH}$ and $-\text{OH}$ functional groups in BCDs as shown in figure 1(c). Morphology and size of prepared BCDs were analysed by HRTEM which indicates the spherical and below 10 nm size of BCDs as shown in figure 1(d).

2.4 Calculation of quantum yield of BCDs [8]

$$Q = Q_R \frac{I_{OD}}{I_{OD}} \frac{n^2}{n_{OD}^2}$$

Where, $Q_R$ = quantum yield of reference, $I$ = deliberate emission intensity is optical density (i.e. measured by UV-Vis spectrophotometer). The quantum yield of BCDs obtained is 4.05 using 350 nm excitation with reference quinine sulphate which has quantum yield 54% in 0.1 M $\text{H}_2\text{SO}_4$($n=1.33$).

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Figure 1. (a) UV-Visible spectra of BCDs, (b) FL excitation spectra, (c) FTIR spectra of BCDs (d) HRTEM image of BCDs
2.5 Interactions of HSA with BCDs
A 2.0 \times 10^{-6} \text{ M} HSA solution was mixed with BCDs solutions ranging from 0.0 – 4.0 \times 10^{-3} \text{ M}. The mentioned solution was incubated for 10 minute for the completion of interaction. Then fluorescence spectra were taken at three different temperatures 295K, 300K and 305K respectively as shown in figure 2(a, b, c).

![Figure 2](image)

**Figure 2.** (a, b, c) FL quenching of HSA by BCDs at three different temperatures, (d) Linear calibration.

3. Results and discussion

3.1 Quenching of fluorescence of HSA by BCDs
Fluorescence spectra of HSA is because of tryptophan (Trp) residue. It is fluorescence is depends upon its intrinsic environment and very sensitive towards different molecules\(^5\). HSA shows fluorescence band at 350 nm when excited upon 278 nm of wavelength. It is observed that fluorescence of HSA was quenched linearly when BCDs solution with increasing concentration was added. The quenching of fluorescence of HSA by BCDs was analysed at three different temperatures using Stern-Volmer equation \(^9\).

\[
\frac{F_0}{F} = 1 + K_{SV} [Q]
\]  
(2)

Where F (FL spectra without BCDs) and F0 (FL spectra with BCDs) \([Q]\) is the concentration of BCDs. By plotting graph between \([Q]\) and \(F_0/F\) the value of was \(K_{SV}\) determined (Table 1).

The apparent binding constant and binding sites can be calculated using following equation \(^10\).

\[
\log \frac{F_0/F}{F} = \log K_a + n \log [Q]
\]  
(3)

By plotting graph between log \((F_0/F)/F\) Vs log \([Q]\), the value of \(K_a\) and \(n\) was calculated. There is 1:1 complex formation between HSA:BCDs. Thermodynamic parameters can be calculated using following formula (Table 2)

\[
\log K_a = - \frac{\Delta H}{2.303RT} + \frac{\Delta S}{2.303R}
\]  
(4)

\[
\Delta G = \Delta H - T\Delta S
\]  
(5)
3.2 Binding site

HSA consists of two domains (II & III) which are homologous and each domain consist of two sub-domain (A & B). To find where the BCDs is binding, site binding fluorescence probes i.e. warfarin for site I and ibuprofen for site II. For the investigation of binding site displacement reactions were performed on the basis of figure 3 (c) and 3(d), on addition of warfarin fluorescence intensity continuously increased and in case of ibuprofen addition fluorescence intensity increased and remains constant, which shows that ibuprofen is further available for binding. Results reveals that BCDs binds to Site I of HSA i.e. domain IIA.

3.3 Conformational change investigation

FTIR spectroscopic studies show that conformational changes of HSA in the presence of BCDs are shown in figure 3(b). HSA shows two amide bands, 1653 cm\(^{-1}\) for C=O stretch, and 1543 cm\(^{-1}\) for C-N stretching and N-H bending. After addition of BCDs there is shifting of spectra is observed up to 1656and 1545 cm\(^{-1}\). These results shows that BCDs affects the C=O, C-N and N-H stretching frequencies [1].

![Figure 3](image)

**Figure 3.** (a) Plot for calculation of binding constant, (b) FTIR spectra of pure HSA and with BCDs, (c, d) Displacement reaction using site probes ibuprofen and warfarin respectively.

| pH value | T(K)  | Ksv\((10^3 \text{ L mol}^{-1})\) | R\(^2\) | S.D.\(^a\) |
|----------|-------|------------------|--------|---------|
| 4.0      | 305   | 2.46             | 0.992  | 0.001   |
| 5.0      | 300   | 2.75             | 0.991  | 0.002   |
| 7.0      | 295   | 2.91             | 0.998  | 0.001   |

Table 1. Stern-Volmer quenching constants Ksv at three temperatures and pH.
Table 2. Stern-Volmer quenching constants $K_{sv}$, associative binding constant and thermodynamic parameters of HSA-BCDs system.

| $T$(K) | $K_{sv}$ ($10^3$ L mol$^{-1}$) | $K_a$ ($10^3$L mol$^{-1}$) | $\Delta H$ (KJ mol$^{-1}$) | $\Delta G$ (KJ mol$^{-1}$) | $\Delta S$ (J mol$^{-1}$) |
|--------|-------------------------------|---------------------------|-----------------------------|-----------------------------|---------------------------|
| 305    | 2.86                          | 4.61                      | -6.34                       | -27.18                      | 68.35                     |
| 300    | 2.96                          | 4.78                      | -7.83                       | -25.83                      | 60.00                     |
| 295    | 3.01                          | 4.85                      | -9.81                       | -28.39                      | 63.00                     |

4. Conclusions
In this work, Boron doped carbon dots have been synthesized successfully in an aqueous medium using microwave treatment technique. Then molecular interaction of BCDs with HSA was investigated by multi-spectroscopic techniques. The results reveal that quenching of intrinsic fluorescence of HSA by BCDs occurred because of the formation of a 1:1 non-fluorescent ground state complex. Displacement experiments suggested that the BCDs bind to the sub domain IIA (sudlow site I) of HSA. Studies of various thermodynamic parameters show that the binding process of BCDs to HSA is spontaneous in nature. Results show that higher concentrations of BCDs can alter the confirmation of serum protein. Based on results obtained one can better understand the potential toxicity of BCDs at molecular level. BCDs-HSA complex may alter the function of the protein by some extent when introduced in the biological systems.

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