Journal of Fluorescence (2019) 29:1191–1200
https://doi.org/10.1007/s10895-019-02431-y

ORIGINAL ARTICLE

Nitrogen and Sulfur Doped Carbon Dots from Amino Acids for Potential Biomedical Applications

Nurettin Sahiner[1,2,3] · Selin S. Suner[1] · Mehtap Sahiner[4] · Coskun Silan[5]

Received: 10 May 2019 / Accepted: 27 August 2019 / Published online: 10 September 2019

Abstract

Nitrogen (N-) and sulfur (S-) doped carbon dots (CDs) were synthesized in a single step in a few min, 1–4 min via microwave technique from five different types of amino acids viz. Arginine (A), Lysine (L), Histidine (H), Cysteine (C), and Methionine (M). These amino acid derived N- and/or S- doped CDs were found to be in spherical shapes with 5–20 nm particle size range determined by Transition Electron Microscope (TEM) images and Dynamic Light Scattering (DLS) measurements. Thermal degradation, functional groups, and surface potential of the CDs were determined by Thermogravimetric Analysis (TGA), FT-IR spectroscopy, and zeta potential measurements, respectively. Although the zeta potential value of Cysteine derived CD (C-CD) was measured as −7.45 ± 1.32 mV, the zeta potential values of A-CD, L-CD, H-CD, and M-CD particles were measured as +2.84 ± 0.67, +2.61 ± 1.0, +4.10 ± 1.50 and + 2.20 ± 0.60 mV, respectively. Amongst the CDs, C- CDs was found to possess the highest quantum yield, 89%. Moreover, the blood compatibility test of CDs, determined with hemolysis and blood clotting tests was shown that CDs at 0.25 mg/mL concentration, CDs has less than 5% hemolysis ratio and higher than 50% blood clotting indexes. Furthermore, A-CD was modified with polyethyleneimine (PEI) and was found that the zeta potential values was increased to +34.41 ± 4.17 mV (from +2.84 ± 0.67 mV) inducing antimicrobial capability to these materials. Minimum Inhibition Concentration (MIC) of A-CD dots was found as 2.5 mg/mL whereas the PEI modified A-CDs, A-CD-PEI was found as 1 mg/mL against *Escherichia coli* ATCC 8739 (gram -) and *Staphylococcus aureus* ATCC 6538 (gram +) bacteria strains signifying the tunability of CDs.

Keywords Fluorescence carbon dots · Amino acid derived CDs · Microwave technique · Blood compatibility · Antimicrobial CDs

Introduction

Carbon dots (CDs) as zero dimensional nanocarbons or carbon particles with <20 nm sizes are considered to replace metal-based quantum dots due their many advantages addition to highly fluorescent properties [1–3]. High photostability, low toxicity, biocompatibility and environmentally friendliness [4, 5] as well as the ease of synthesis and with tunable fluorescence and optical properties [6–8] make them capable materials with great potential in biomedical use and optoelectronic materials including sensor [9, 10], biosensor [11, 12], bioimaging [13], medical diagnostic [14], photocatalysis [15], electrocatalysis [16], and so on [1]. Carbon dots have been prepared from various carbon sources and can be doped with various element to render additional properties employing different synthesis method such as bottom-up method by hydrothermal/solvothermal, thermal pyrolysis, microreactor, oil-bath, and microwave processes [17]. To increase the usage area and enhance the physicochemical properties, during CDs synthesis new material introduction containing nitrogen (N), phosphorus (P), sulfur (S) and boron (B) can done to obtain doped CDs [18, 19]. Especially, N- or/and S-doped CDs can be prepared employing various amino acids that possess -COOH, -NH and -SH groups side groups produce

✉ Nurettin Sahiner
sahiner71@gmail.com; nsahiner@health.usf.edu

[1] Faculty of Sciences and Arts, Chemistry Department, Canakkale Onsekiz Mart University, Canakkale, Turkey

[2] Nanoscience and Technology Research and Application Center (NANORAC), Terzioglu Campus, 17100 Canakkale, Turkey

[3] Department of Ophthalmology, Morsani School of Medicine, University of South Florida, 12901, Bruce B Downs Blvd., MDC 21, Tampa, FL 33612, USA

[4] Fashion Design, Canakkale Applied Science, Canakkale Onsekiz Mart University, Terzioglu Campus, 17100 Canakkale, Turkey

[5] School of Medicine, Department of Pharmacology, Canakkale Onsekiz Mart University, Terzioglu Campus, 17100 Canakkale, Turkey

materials that are non-toxic, biocompatible and has great meaning for biomedical applications [20–24].

It is very well-known that materials with florescent properties such as organic dyes and metal-based quantum dots (Q-dots) possess undesirable properties that can chemically react with the biological medium and induce toxicity for living organism. Moreover, organic chemical dyes with fluorescence property may not keep their fluorescence properties long enough, can be toxic, reactive and expensive. Also, some of the toxic metal-based Q-dots necessitate surface modification to make them non-toxic and non-reactive also render adverse effects on the florescence intensity and biomedical usage of these materials [17].

In this study, amino acids such as Arginine (A), Lysine (L), Histidine (H), Cysteine (C), and Methionine (M) with different numbers of -NH and/or -SH were used as source materials for (N) and sulfur (S) doped CDs synthesis via microwave method in very short times, ~1–4 min. The optical and florescent properties of the prepared CDs were measured via UV-Vis and Fluorescence spectroscopies, and the quantum yields were also calculated. Employing hemolysis and blood clotting tests on fresh blood, the blood compatibility of the prepared CDs was investigated in vitro. Thus, 5 types of amino acid based non-toxic and biocompatible CDs were investigated as potential biomaterials with strongly photoluminescence characteristics. Furthermore, antimicrobial properties of A-CD and its modified form with cationic polyethyleneimine (PEI) as A-CD-PEI against *Escherichia coli* ATCC 8739 (gram -) and *Staphylococcus aureus* ATCC 6538 (gram +) were also determined with macro-dilution test.

Experimental

Materials

Citric acid monohydrate (Carlo Erba, >99%) as a carbon source and L-Arginine (A, Sigma Aldrich, >98%), L-Lysine (L, Sigma Aldrich, >97%) and L-Histidine monohydrochloride monohydrate (H, Sigma Aldrich, >98%), L-Cysteine (C,Sigma Aldrich, >97%) and DL-Methionine (M, Sigma Aldrich, >99%) as amino acids were used in the synthesis of amino acid derived N or/and S doped CDs. Polyethyleneimine (PEI, 50%, Mn: ~1200–1300), epichlorohydrine (ECH, >99%), sodium hydroxide (NaOH, 98%) and hydrochloric acid (HCl, 36.5–38%), calcium chloride (99.9%) and sodium chloride (NaCl, 99%) were purchased from Sigma-Aldrich. Pyrene (>99%, Merck) and quinine (suitable for fluorescence, anhydrous, >98, Sigma-Aldrich) were used as fluorescence standards. Nutrient agar (Merck) and nutrient broth (Merck) were in antimicrobial studies. Ethanol (99%, Birkim) and acetone (99%, BRK) were used as received. *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538 strains were obtained from the Microbiology Department of the School of Medicine at Canakkale Onsekiz Mart University. All aqueous solutions were prepared with ultra-pure distilled water, 18.2 M.Ω.cm (Millipore-Direct Q UV3).

Synthesis of Amino Acid Derived N or/and S Doped CDs

N or/and S doped CDs were prepared according to the reported processes with slight modification [25, 26]. In brief, 0.2 g citric acid were dissolved in 2.5 mL of DI water and 0.1 g of amino acids, A, L, H, C or M was added to this solution and mixed for 15 min at 500 rpm. These solutions were placed in microwave operating at 700 W for 1, 2 or 4 min to synthesize N or/and S doped CDs. The obtained samples depending on the microwave exposure time became viscous solution and/or foamy solids that were mixed with 2 mL DI water and sonicated for 20 s. Then, these suspensions were filtered with 0.2 μm filters to eliminate of the carbon particles into the solution. These suspensions were put into dialysis membrane and washed into DI water for 4 h. The prepared CDs were precipitated in excess amounts of acetone by centrifugation at 35544 *g* for 10 min and dried with heat gun.

Modification of Amino Acid Derived CDs

A-CDs weighing 0.4 g were suspended within 10 mL of NaOH solution adjusted at pH 12 and mixed for 15 min at 500 rpm at ambient temperature. Then, 0.8 mL of ECH, coupling agent was added to this solution. After 1 h, 0.8 mL of PEI as a modifying agent was transferred into the reaction solution drop by drop and mixing for 1 h more under the same reaction conditions. PEI modified A-CD as A-CD-PEI were precipitated with acetone and washed with acetone 2 times by centrifugation at 35544 *g* for 10 min and dried with heat gun.

Characterization of Amino Acid Derived CDs

The visualization or CDs were done with Transmission Electron Microscope (TEM, Jeol JEM-1220) by using CDs suspension in ethanol and one drop of this suspension was placed on TEM grid and dried at ambient temperature before imaging operating with 80 kV.

Hydrodynamic diameter and zeta potential of CDs were determined by Dynamic Light Scattering (DLS, Brookhaven Ins. Corp., 90Plus/BIMAS) with 35 mW solid state laser detector at an operating wavelength of 658 nm. Zeta potential measurements of the CDs were evaluated by using zeta potential analyzer (Brookhaven Inst. Corp., BIC ZetaPlus) at different pH solutions in DI water.

The functional group analysis of CDs were done using with FT-IR spectroscopy (Nikolet IS10, Thermo) between 4000

and 650 cm^{-1} wavelength with 4 cm^{-1} resolution using ATR technique.

Thermal stabilty of CDs were measured using Thermogravimetric Analyzer (TGA, SII TG/DTA 6300). Nearly, 5 mg of sample was placed into ceramic crucibles and heated from 50 to 1000 °C with 10 °C/min heating rate under a dry flow of N_2 of 100 mL/min.

Optical properties of the prepared CDs were determined by means of UV-Vis spectroscopy (T80+ UV/VIS spectrometer, PG Instrument Ltd) and Fluorescence Spectroscopy (Lumina Fluorescence Spectrometer, Thermo Scientific) at room temperature.

Quantum Yield (QY) of Amino Acid Derived CDs

The QY of CDs was measured by using established process [27]. Pyrene in ethanol and quinine in 0.5 M H_2SO_4 in DI water were used as standards that are known for QY values as 53% at 313 nm excitation wavelength for pyrene, and 54% at 345 nm excitation wavelength for quinine sulphate. The concentration of amino acid derived CDs suspension in DI water were adjusted between 0.05 and 0.01 absorbance values at 313 or 345 nm wavelength. The relative QY was found using the below Eq. 1:

$$QY_{CDs}(\%) = QY_{st}(Grad_{CDs}/Grad_{st})\left(\eta^2{}_{CDs}/\eta^2{}_{st}\right) \quad (1)$$

Where Grad is the slope from the plot of the integrated of photoluminescence (PL) intensities from 320 to 600 nm wavelength versus absorbance values and η is the refractive index of the solvent for CDs suspension solution as DI water (η = 1.33) and the standard solution as ethanol (η = 1.36) or 0.5 M H_2SO_4 in water (η = 1.76).

Blood Compatibility of Amino Acid Derived CDs

Blood compatibility study was approved by the Human Research Ethics Committee of Canakkale Onsekiz Mart University (KAEK 2017/07–06). Fresh blood was taken from healthy volunteers and placed into EDTA containing hemogram tubes. The blood compatibility of the CDs was determined with hemolysis and blood clotting tests.

Hemolysis Test

CDs about 0.25 mg/mL suspension was prepared in 10 mL of 0.9% saline solution and 0.2 mL of diluted blood, which is prepared at 2:2.5 (v:v) ratio of fresh blood:0.9% saline solution was slowly added to the sample containing saline solution. These suspensions were put into shaker bath under gentle shaking at 37 °C for 1 h. Then, these suspensions were centrifuged at 100 *g* for 5 min and the absorbance value of supernatant solutions was measured with UV-Vis spectroscopy at 542 nm wavelength to determine the absorbance of hemoglobin. As a positive control (PC) and negative control (NC), 0.2 mL of diluted blood was dispersed into 10 mL DI water and 0.9% saline solution, respectively. Hemolysis ratio of CDs were calculated using Eq. 2.

$$\text{Hemolysis ratio}\% = 100\ (A_{CDs}\text{-}A_{NC}/A_{PC}\text{-}A_{NC}) \quad (2)$$

Where A_{CDs}, A_{NC}, and A_{PC} are the absorbance values of the CDs containing blood solutions, negative control, and positive control, respectively.

Blood Clotting Test

For blood clotting test, 100 μL of 25 mg/mL CDs suspension were interacted with 0.27 mL of diluted blood samples that was prepared at 3:0.24 (v:v) ratio of fresh blood:0.2 M $CaCl_2$ solution. These samples was in a shaker with gently shaking at 37 °C for 10 min. Then, 10 mL DI water was slowly added onto these sample that is interacted with blood suspension and centrifuges at 100 *g* for 30 s. The supernatant solution was taken and diluted with 40 mL of DI water, and placed into shaking bath under slowly shaking at 37 °C for 1 h. As a control group 0.25 mL of fresh blood was dispersed in 50 mL DI water. Then, the absorbance values of these solutions were measured by UV-Vis spectroscopy at 542 nm wavelength and blood clotting index was calculated using Eq. 3.

$$\text{Blood clotting index} = 100\ (A_{CDs}/A_{control}) \quad (3)$$

Where A_{CDs} and $A_{control}$ are the absorbance values of the CDs contacted blood solutions and only diluted blood solution as a control, respectively.

Antimicrobial Effects of the CDs

Antimicrobial capabilities of A-CD and A-CD-PEI were investigated by using macro-dilution test against *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538 bacteria strains. First, CDs were sterilized via UV irradiation at 420 nm for 1 min, and five different amounts of CD, 5, 10, 25, 50 and 100 mg was put into 10 mL of nutrient broth (NB). Then, 100 μL of nearly 1×10^8 CFU/mL (colony forming unit) bacterial suspension that was adjusted with McFarland 0.5 standard was added to the NB solutions and incubated in shaker at 35 °C for 18–24 h. The minimum inhibition concentration (MIC) value was determined as the lowest concentration of the medium that no visible growth was observed. Then, 100 μL of each suspension were inoculated into nutrient agar and incubated at 35 °C for 18–24 h. The growing colonies were counted and minimum bactericidal concentration (MBC) value was determined as the lowest concentration of CD that 99.9% of microorganisms can be eliminated.

Results and Discussion

Molecular structure of the starting materials along with the employed synthesis techniques as well as the reaction times are the most important parameters to be considered in preparation of CDs with higher quantum yields. Here, amino acid derived CDs were prepared employing citric acid with different amino acids having different numbers of -NH and -SH functional groups in a very short times, 1–4 min via microwave technique. Fig. 1a and b illustrates the chemical structure and the synthesis scheme and the chemical structures of C-CDs.

As amino acids have amino and carboxylic acid groups which bounded with different functional group containing -R side chains that enables the inclusion of these functional groups in CDs. As shown in Fig. 1a, the molecular structure of A, L and H amino acids can provide N atoms whereas C and M amino acids can provide N and S atoms to the resultant CDs. Upon mixing these molecules with citric acid as another carbon sources and upon exposing this mixture to microwave irradiation, N and/or S-doped CDs were successfully synthesized in very short time, 1–4 min as illustrated in Fig. 1b. The CD generation is assumed in two steps; one of which is the reaction of carboxylic groups of citric acid with the -NH and -SH groups of amino acids via condensation polymerization and then the esterification of citric acid with amino acids. In the second step during irradiation is the carbonization of these

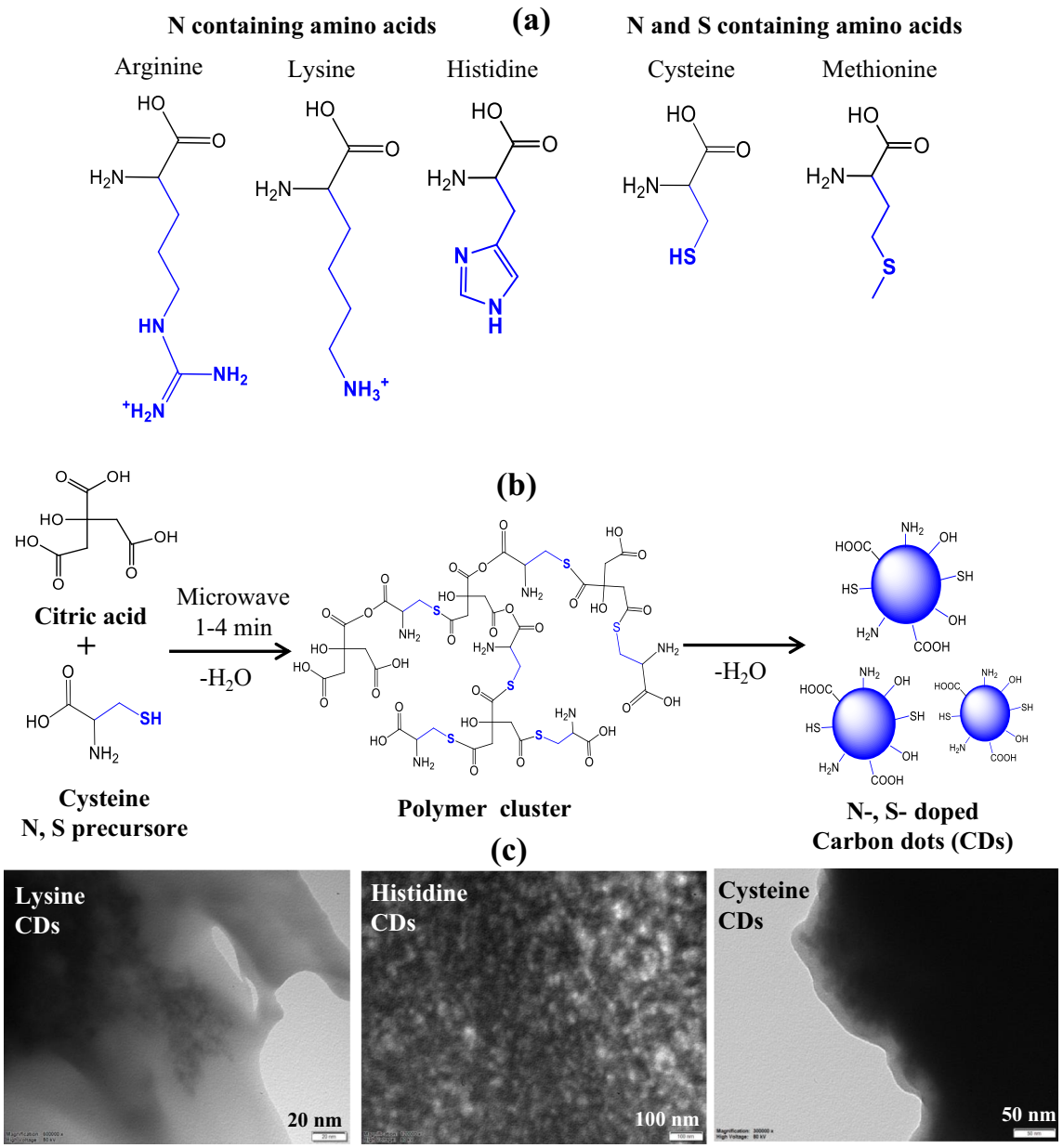

Fig. 1 **a** Molecular structure of prepared amino acids, **b** Schematic representation of N-, S-doped CDs preparation with citric acid as a carbon source and cysteine as a S and N doping source. **c** TEM images of amino acid derived CDs

polymer clusters by total dehydration to generate N and/or S doped CDs under microwave exposure [23, 27].

Therefore, this very fast synthesis of A, L, H, C or M derived N- and/or S- doped CDs using microwave technique can offer great advantage for biomedical applications. The visualization of these CDs was also determined by TEM images as show in Fig. 1c. The TEM images shown for L-, H-, and C- based CD clusters are in spherical shapes at about 20 nm size range. The particle sizes and zeta potential values of the prepared amino acid derived CDs were also investigated via DLS and zeta potential measurements and the results were summarized in Table 1. The size distribution of the CDs found as 11 ± 4, 17 ± 2, 6 ± 5, 10 ± 7 and 9 ± 5 nm for A, L, H, C and M, respectively.

According to the DLS measurements, the size of the amino acid derived CDs were found to be between 5 and 20 nm. In addition, zeta potential of A, L, H, C, and M CDs were measured as +2.84 ± 0.67, +2.61 ± 1.01, +4.10 ± 1.50, −7.45 ± 1.31 and + 2.20 ± 0.60 mV, respectively. Only C CD possess a negative charge because of the existence of -SH groups that has the lowest iso electric point, 5.07 in comparison to the other amino acids used in CDs preparation.

The surface functionality of amino acid derived CD was also confirmed by FT-IR spectroscopy and the corresponding spectrum for each of the prepared CDs were demonstrated in Fig. 2a. The broad band at 3400–3100 cm^{-1} was attributed to C-N and = C-O -OH and -NH stretching vibrations for all the CDs. The peaks at 2948 and 2870 cm^{-1} and 1701 cm^{-1} were assigned to the stretching vibrations of -CH_2, C-H and C=O groups coming from the newly formed CDs structure. The peaks at 1662–1625 cm^{-1}, 1395, and 1217 cm^{-1} can be attributed to C=N, C-N, and = C-O stretching vibrations as seen in all N-doped CDs.

Furthermore, the specific peaks at 1217–1170 cm^{-1} can be ascribed to aromatic amine groups of H derived CDs. In addition, N and S-doped CDs have S-H peaks at 2560 cm^{-1} due to the side chain of C and the stretching vibrations at 1200 and 1048 cm^{-1} attributed to S-C groups of C and M derived CDs. These results confirm that the resultant CDs can have N and/or S doping and have -OH, -COOH, -NH, -SH, and -SC functional groups depending on the used amino acid precursors.

Table 1 Particle sizes and zeta potential values of amino acid derived CDs and modified CDs with PEI

CarbonDots (CDs)	Particlesizes (nm)	Zeta Potential (mV)
A-CD	11 ± 4	+2.84 ± 0.67
L-CD	17 ± 2	+2.61 ± 1.01
H-CD	6 ± 5	+4.10 ± 1.50
C-CD	10 ± 7	−7.45 ± 1.32
M-CD	9 ± 5	+2.20 ± 0.60
A-CD-PEI	31 ± 7	+34.41 ± 4.17

Thermal degradation profile of N doped L-CDs, and N and S-doped C-CDs were determined by thermogravimetric analysis between 50 and 1000 °C as shown in Fig. 2b. As can be seen in the thermogram of N-doped Lysine CDs, the first degradation was between 140 and 174 °C with 8.1% weight loss, and the main degradations were observed in 75–220 °C range with 16.5% weight loss and the last one was observed at 225–652 range °C with 97.7% weight losses. Furthermore, the thermal degradation of N, S-doped Cysteine CDs have two main degradation steps between 161 and 276 °C and 400–584 °C with 38% and 98% weight losses. It is obvious that thermal stability of N-doped and N, S-doped CDs are about the same with slightly differences in200–600 °C range because of different starting materials and doping groups.

The optical properties of N and/or S-doped CDs were evaluated using UV-Vis and Fluorescence spectroscopies as demonstrated in Fig. 3a and b, respectively. As can be seen in the UV-Vis spectrums, a strong absorption shoulder at about 220–230 nm attributed to π-π* electron transition of aromatic sp^2 domains of C=C and C=N bonds for all types of CDs. In addition, the peaks at 290 nm are due to the n-π* electron transition from C=O bonds for L-CDs. N, S-doped C-CDs have three absorption peaks at 210 and 245 nm due to π-π* electron transition of C=C and 350 nm attributed to n-π* electron transition of C=O and C=S bonds [8, 23]. All types of CDs have a slightly absorption at 350 nm due to n-π* electron transition of C=O groups, but only C-CDs has strong broad band at 350 nm because of the n-π* electron transition of C=S bonds in addition of C=O bonds. In accord with the literature, the broad absorption band between 300 and 400 nm based on the trapping of excited state energy by the surface state that demonstrates strong fluorescent properties [8, 28]. Therefore, the use of amino acid in the preparation of non-toxic CDs with high fluorescent properties offer great avenue in biomedical application and even can replace of the fluorescence dyes and/or metal based quantum dots (Q-dot) such as CdS because of the very well-known toxic disadvantages of these materials.

The photoluminescence (PL) emission was depend on excitation wavelength of CDs. Upon the excitation at 280 to 360 nm of CDs, the maximum emission peaks of A-, L-, H-, C- and M-CD was measured. The PL intensity of these CDs at 330 nm excitation wavelength were shown in Fig. 3b were measured at 430 nm, 430 nm, 433 nm, 420 nm, and 407 nm, respectively.

The digital camera images of the amino acid derived CDs under white light, 254 nm UV light and 366 nm UV light were shown in Fig. 3c. It is obvious that all the CDs have the bright blue luminescence under UV light illumination at 254 nm, but only C-CDs were luminescence at 366 nm UV light. Among the all prepared CDs, the highest PL efficiency were shown in N- and S-doped C-CDs. It is well known that the synthesis method, reaction temperature and reaction times play

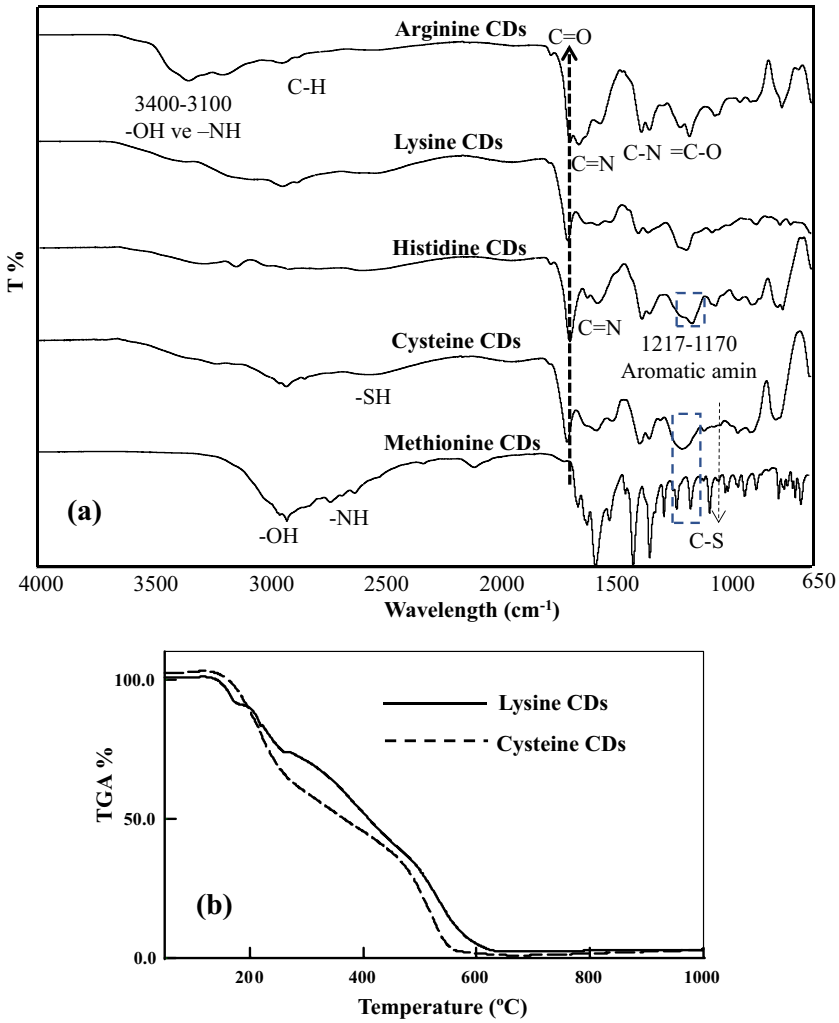

Fig. 2 **a** FT-IR spectra, and **b** termal degradation analysis (TGA) of Lysine and Cysteine derived CDs

significant role on the PL efficiency and QY of the prepared CDs [17, 29]. Here, the CDs were prepared by using microwave irradiation at 1, 2, and 4 min reaction times and their quantum yield (QY) were calculated according to UV-Vis absorbance spectra and integrated with the photoluminescence (PL) intensities between 320 and 600 nm wavelength using pyrene and quinine sulfate as reference materials at 313 nm 345 nm excitation wavelength, respectively. The relative QY of A-, L-, H-, C- and M-CDs prepared in 1 min microwave irradiation were calculated as 29 ± 3%, 75 ± 6%, 23 ± 2%, 71 ± 4%, and 12.9 ± 0.8%, respectively according to pyrene standard at 313 nm excitation wavelength as shown in the Fig. 3d. Using quinone sulphate as a reference material at 345 nm excitation wavelength, the relative QY of N- and/or S-doped CDs were calculated as 3.9 ± 0.4%, 4.2 ± 1.9%, 2.8 ± 0.2%, 89.5 ± 2.3%, 2.5 ± 0.6% for A-, L-, H-, C- and M-CDs, respectively. Only C-CD was found suitable for quinone sulfate standard with highly quantum yield upon excitation at 345 nm, whereas the other types of CDs was shown effectively fluorescence efficiency at 313 nm excitation wavelength using pyrene as standard in QY calculations. The QY of all QDs prepared at 1- and 2-min irradiation times were about the same except C-QDs that showed significant increase to about 71% from 41% by increasing the synthesis time to 2 min from 1 min. All the other CDs showed very slight increase or decrease upon 4 min microwave preparation methods except M-QDs that the QY was increased to 20% from 13% upon increasing the microwave irradiation from 2 to 4 min. As the all the used amino acids have different functional groups, the increase in the synthesis time may cause depletion of fluorescence groups during the carbonization process by excessive irradiation time [29], these outcomes are reasonable. The fluorescence properties of CDs significantly depend on the carbon content and functionalities of precursor materials. Especially, N or/and S atom containing CDs have the higher QY values compare with undoped materials. These results supported by the fact that N- doped CDs have strong fluorescence effects in blue shift, whereas N- and S-doped C-CDs was shown wide range of excitation wavelength with the highest QY values. Moreover, S groups containing M-CDs was not found to be effective photoluminescent material in comparison with the -SH groups of C-CDs. It was reported that the preparation of CDs using

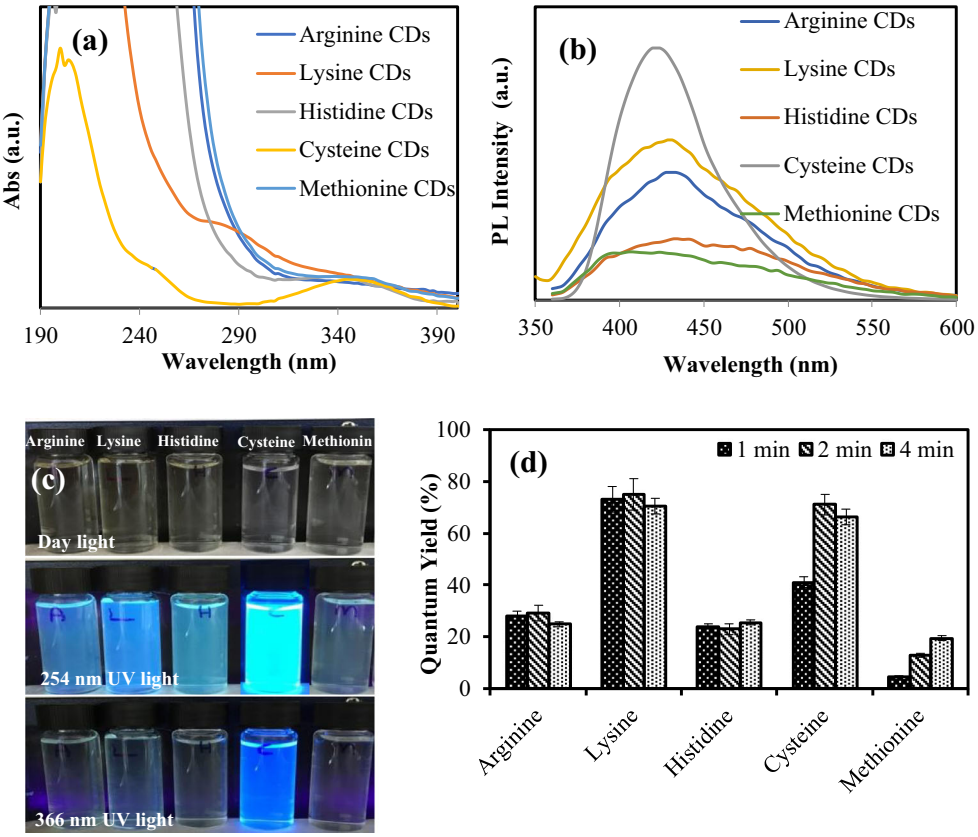

Fig. 3 **a** UV-Vis absorption spectra, and **b** Fluorescence maximum emission spectra at 330 nm excitation wavelength of amino acid derived CDs. **c** Digital camera image of amino acid derived CDs under day light, UV light at 254 nm and 365 nm wavelength. **d** Quantum Yields (QY) of amino acid derived CDs prepared at different time course under microwave exposure measured against pyrene as a standard fluorescent probe

different amino acid via hydrothermal methods took about 6 h to synthesize [22] with QY values between 10 and 38%. As CDs are very useful materials for the conjugation of peptides, nucleotides and anticancer drugs that can have great potential in clinical sample analysis, early diagnosis of sickness and sensor applications [20]. Therefore, the fast and facile synthesis CDs with high QY could be very important in biomedical use of these materials. Only, A-QDs with high QY was reported with microwave synthesis in the literature, and even the quantum yield of that material is rather low [22]. Therefore, using different amino acids such as A, L, H, C, and M, 5 different N- and/or S-doped CDs were readily synthesized here in about 2 min via microwave methods with high fluorescence behavior upon comparison literature may pave great opportunity in real biomedical applications [20–22].

In addition to various important features such as biocompatibility, non-immunogenicity and non-toxicity, blood compatibility is also another important features of biomaterials that need to be assessed in various biomedical applications. Therefore, hemolysis ratio and blood clotting index of the amino acid derived CDs were determined at 0.25 mg/mL concentration by contacting with fresh blood and their results were demonstrated in Fig. 4.

According to the hemolysis test, A- and L-CDs were found blood compatible with 3.15 ± 1.3% and 2.07 ± 1.84% hemolysis ratio, whereas H-, C- and M-CDs were

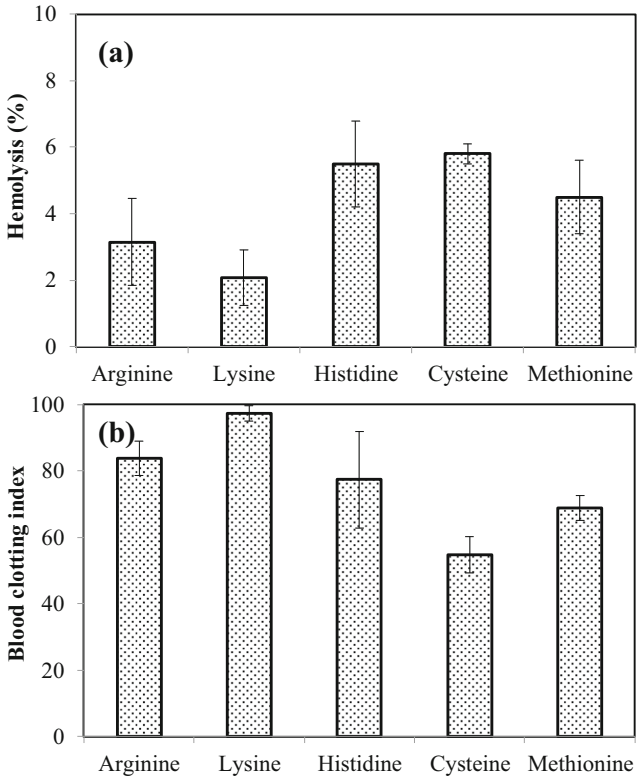

Fig. 4 **a** Hemolysis ratio %, and **b** blood clotting indexes of amino acid derived CDs at 0.25 mg/mL concentration

found slightly hemolytic with 5.5 ± 2.3%, 5.8 ± 0.3%, and 4.5 ± 1.1% hemolysis ratios at 0.25 mg/mL concentration. Materials with <5% hemolysis ratio is considered as blood compatible whereas materials with higher blood clotting index is considered as blood compatible. Blood clotting index results of the CDs were shown very similar results with hemolysis results as A- and L-CDs were not shown very important effect on the thrombogenic activity of the blood with 83.7 ± 5.1 an 97.2 ± 4.0 blood clotting indexes, whereas H-, C- and M-CDs can clot the blood with 67.7 ± 19.5, 54.7 ± 5.4, and 68.7 ± 3.6% blood clotting indexes, respectively at 0.25 mg/mL concentration. Therefore, these amino acid-based CDs can be presumed blood compatible below 0.25 mg/mL concentration for potential blood contacting applications.

To further affirm the versatility of amino acid derived CD and impart new properties, e.g., attest antimicrobial properties, A-CDs were modified with polyethyleneimine (PEI), a well-known effectively antimicrobial material cationic charge. The schematic representation of modification reaction was illustrated in Fig. 5a. A-CDs having -OH, -COOH, -NH and -NH_2 groups on the surface was treated can be further modified to conjugate PEI. Therefore, A-CDs were treated with NaOH and then reacted with epichlorohydrin (ECH) as coupling agent.

Upon modification of A-CDs with ECH, PEI that has abundant number of -NH_2 groups can readily react with ECH on the surface of A-CD to generate A-CD-PEI. Consequently, zeta potential values of A-CDs was increased from +2.84 ± 0.67 mV to +34.41 ± 4.17 mV for A-CD-PEI as given in Table 1. Additionally, the size of this modified CD, A-CD-PEI is increased significantly due to the surface coating of PEI from 11 ± 4 to 31 ± 7 nm. The surface modification is further corroborated with FT-IR analysis and the corresponding FT-IR spectra are demonstrated in Fig. 5b. It is obvious in the spectrum that the PEI modified A-CDs have strong bands at 3400–3100 cm^{-1}, 2948–2870 cm^{-1}, and 1395 cm^{-1} because of the stretching vibrations of -NH, C-H, C-N and -NH groups and their intensities were increased significantly due to newly attached PEI groups. The rest of stretching frequencies in A-CD-PEI are in accordance with PEI FT-IR peaks conforming the success of modification reactions.

To evaluate the effects of the modification reaction, antimicrobial properties of A-CD and A-CD-PEI were determined against *Escherichia coli* ATCC 8739 (gram -) and *Staphylococcus aureus* ATCC 6538 (gram +) bacteria strains, and the Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of A-CD and A-CD-PEI particles were measured and compared in Table 2.

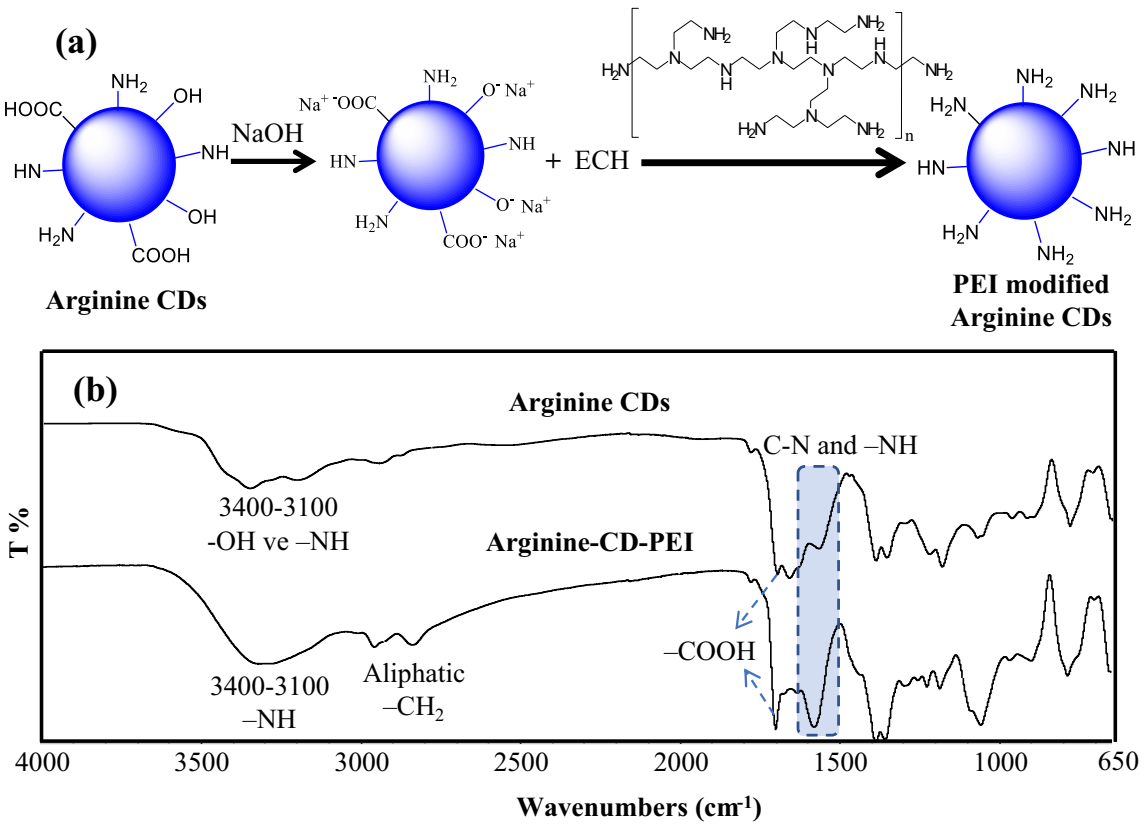

Fig. 5 **a** The Schematic representation of modification reaction of A-CDs with polyethyleneimine (PEI) by using epichlorohydrin (ECH) as coupling agent, and **b** FT-IR spectra of A-CD and A-CD-PEI

Table 2 Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values of A-CD and modified A-CD-PEI particles against *Escherichia coli* ATCC 8739 (gram -) and *Staphylococcus aureus* ATCC 6538 (gram +) bacteria strains

Carbon Dots (CD)	MIC (mg/mL)		MBC (mg/mL)	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*
A-CD	2.5	2.5	–	10
A-CD-PEI	1	1	2.5	5

It is obvious that the MIC values of A-CDs was significantly reduced upon PEI modification which is 2.5-fold reduction for both bacteria from 2.5 to 1 mg/mL against *E. coli* and *S. aureus*. Furthermore, the MBC could only be determined against *S. aureus* at 10 mg/mL concentration for A-CD particles. Interestingly, the MBC values of A-CD-PEI was significantly reduced for S. aureus from 10 to 5 mg/mL. Moreover, the MBC value against *E. coli* for A-CD cannot be determined as there was no antibacterial activity against this microorganism, *however*, upon PEI modification, A-CD-PEI revealed strong antimicrobial susceptibility against E. coli. These results suggest that amino acid derived CDs with chemical modification can reveal another important application of these materials as antimicrobial materials as another potential biomedical applications.

Conclusions

Here, we reported an easy, fast and facile preparation method of N-, and/or S-doped amino acid such as A-, L-, H-, C- and M- derived CD in a single step via microwave irradiation methods in 2 min for biomedical applications. The following significant outcome was attained;

- High photoluminescence efficiencies similar to fluorescence dyes with high quantum yields such as 29 ± 3%, 75 ± 6%, 23 ± 2%, 71 ± 4%, and 12.9 ± 0.8% for L-, H-, C- and M-CDs, were measured suggesting the potential bioimaging applications of these amino acid derived CDs.
- A- and L-CDs were found to be blood compatible with less hemolysis and highest blood clotting indexes up to 0.25 mg/mL concentration whereas H-, C- and M-CDs can be considered blood compatible at <0.25 mg/mL concentration.
- Because of the many functional groups of the prepared amino acid-based CD, the chemical modification was shown the effective was to introduce new functionality and properties to these CDs. For example, the zeta potential value of +2.84 ± 0.67 mV A- CDs were increased to +34.41 ± 4.17 mV with PEI modification (A-CD-PEI) that supplemented an antimicrobial property for this blood comparable CDs against *Escherichia coli* ATCC 8739 (gram -) and *Staphylococcus aureus* ATCC 6538 (gram +) bacteria strains.

Consequently, these amino acid derived CD are versatile and offer great avenue for the monitoring and/or deciphering various biological events such as determination the action of interaction of drug molecules with cell or micro organism and so on.

Acknowledgments This work is supported by the Scientific Research Commission of Canakkale Onsekiz Mart University (COMU BAP, TSA-2018-2457).

References

1. Yuan F, Li S, Fan Z, Meng X, Fan L, Yang S (2016) Shining carbon dots: synthesis and biomedical and optoelectronic applications. Nano Today 11:565–586
2. Sun YP, Zhou B, Lin Y, Wang W, Fernando KAS, Pathak P, Meziani MJ, Harruff BA, Wang X, Wang H, Luo PG, Yang H, Kose ME, Chen B, Veca M, S-Y X (2006) Quantum-sized carbon dots for bright and colorful photoluminescence. J Am Chem Soc 128(24):7756–7757
3. Li H, Kang Z, Liu Y, Lee S-T (2012) Carbon nanodots: synthesis, properties and applications. J Mater Chem A 22:24230–24253
4. Wang D, Wang X, Guo Y, Liu W, Qin W (2014a) Luminescent properties of milk carbon dots and their Sulphur and nitrogen doped analogues. RSC Adv 4:51658–51665
5. Ponomarenko LA, Schedin F, Katsnelson MI, Yang R, Hill EW, Novoselov KS, Geim AK (2008) Chaotic Dirac billiard in graphene quantum dots. Science 320:356–358
6. Song Z, Quan F, Xu Y, Liu M, Cui L, Liu J (2016) Multifunctional N, S co-doped carbon quantum dots with pH- and thermo-dependent switchable fluorescent properties and highly selective detection of glutathione. Carbon 104:169–178
7. Li X, Lau SP, Tang L, Jic R, Yang P (2014) Sulphur doping: a facile approach to tune the electronic structure and optical properties of graphene quantum dots. Nanoscale 6:5323–5328
8. Xue M, Zhang L, Zou M, Lan C, Zhan Z, Zhao S (2015) Nitrogen and sulfur co-doped carbon dots: a facile and green fluorescence probe for free chlorine. Sensors Actuators B Chem 219:50–56
9. Dong Y, Cai J, You X, Chi Y (2015) Sensing applications of luminescent carbon based dots. Analyst 140:7468–7486
10. Dong Y, Li G, Zhou N, Wang R, Chi Y, Chen G (2012) Graphene quantum dot as a green and facile sensor for free chlorine in drinking water. Anal Chem 84:8378–8382
11. Zhang L, Zhang Z-Y, Liang R-P, Li Y-H, Qiu J-D (2014a) Boron-doped graphene quantum dots for selective glucose sensing based on the "abnormal" aggregation-induced photoluminescence enhancement. Anal Chem 86:4423–4430
12. Ding C, Zhu A, Tian Y (2014) Functional surface engineering of C-dots for fluorescent biosensing and in vivo bioimaging. Acc Chem Res 47:20–30
13. Mehta VN, Jha S, Kailasa SK (2014) One-pot green synthesis of carbon dots by using *Saccharum officinarum* juice for fluorescent imaging of bacteria (*Escherichia coli*) and yeast (*Saccharomyces cerevisiae*) cells. Mater Sci Eng C 38:20–27
14. Cheng L, Li Y, Zhai X, Xu B, Cao Z, Liu W (2014) Polycation-b-Polyzwitterion copolymer grafted luminescent carbon dots as a

multifunctional platform for serum-resistant gene delivery and bioimaging. ACS Appl Mater Interfaces 6:20487–20497
15. Fernando KAS, Sahu S, Liu Y, Lewis WK, Guliants EA, Jafariyan A, Wang P, Bunker CE, Sun YP (2015) Carbon quantum dots and applications in photocatalytic energy conversion. ACS Appl Mater Interfaces 7:8363–8376
16. Liu Q, Zhang S, Dai L, Li LS (2012) Nitrogen-doped colloidal graphene quantum dots and their size dependent Electrocatalytic activity for the oxygen reduction reaction. J Am Chem Soc 134: 18932–18935
17. Sagbas S, Sahiner N (2019) Nanocarbon and its composites. In: Khan A, Jawaid M, Inamuddin DR, Asiri AM (eds) Chapter 22: Carbon dots: Preparation, Properties and Application, 1st edn. Elsevier, Woodhead
18. Zhang B-X, Gao H, Li X-L (2014b) Synthesis and optical properties of nitrogen and sulfur co-doped graphene quantum dots. New J Chem 38:4615–4621
19. Wang C, Sun D, Zhuo K, Zhang H, Wang J (2014b) Simple and green synthesis of nitrogen-, sulfur-, and phosphorus-co-doped carbon dots with tunable luminescence properties and sensing application. RSC Adv 4:54060–54065
20. Wang Z, Xu C, Lu Y, Chen X, Yuan H, Wei G, Ye G, Chen J (2016) Fluorescence sensor array based on amino acid derived carbon dots for pattern-based detection of toxic metal ions. Sensors Actuators B Chem 241:1324–1330
21. Zou S, Hou C, Fa H, Zhang l MY, Dong L, Li D, Huo D, Yang M (2017) An efficient fluorescent probe for fluazinam using N, S co-doped carbon dots from l-cysteine. Sensors Actuators B Chem 239: 1033–1041
22. Philippidis A, Stefanakis D, Anglos D, Ghanotakis D (2013) Microwave heating of arginine yields highly fluorescent nanoparticles. J Nanopart Res 15:1414
23. Zeng YW, Ma DK, Wang W, Chen JJ, Zhou L, Zheng YZ, Yub K, Huang SM (2015) N, S co-doped carbon dots with orange luminescence synthesized through polymerization and carbonization reaction of amino acids. Appl Surf Sci 342:136–143
24. Karfa P, Roy E, Patra S, Kumar S, Tarafdar A, Madhuri R, Sharma PK (2015) Amino acid derived highly luminescent, heteroatom-doped carbon dots for label-free detection of Cd^{2+}/Fe^{3+}, cell imaging and enhanced antibacterial activity. RSC Adv 5:58141–58153
25. Zhu H, Wang X, Li Y, Wang Z, Yang F, Yang X (2009) Microwave synthesis of fluorescent carbon nanoparticles with electrochemiluminescence properties. Chem Commun (34): 5118–5120
26. Kudr J, Richtera L, Xhaxhiu K, Hynek D, Heger Z, Zitka O, Adam V (2017) Carbon dots based FRET for the detection of DNA damage. Biosens Bioelectron 92:133–139
27. Gao F, Ma S, Li J, Dai K, Xiao X, Zhao D, Gong W (2017) Rational design of high quality citric acid-derived carbon dots by selecting efficient chemical structure motifs. Carbon 112:131–141
28. Liu T, Li N, Dong JX, Zhang Y, Fan YZ, Lin SM, Luo HQ, Li NB (2017) A colorimetric and fluorometric dual-signal sensor for arginine detection by inhibiting the growth of gold nanoparticles/carbon quantum dots composite. Biosens Bioelectron 87:772–778
29. He G, Shu M, Yang Z, Ma Y, Huang D, Xu S, Wang Y, Hu N, Zhang Y, Xu L (2017) Microwave formation and photoluminescence mechanisms of multi-states nitrogen doped carbon dots. Appl Surf Sci 422:257–265