



Fluorescent Carbon Dots Prepared from Hazelnut Kohl as an Affordable Probe for Determination of Dopamine

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Abstract

In this investigation, a simple, green and facile fluorescence method using carbon dots (CDs) of hazelnut kohl is described for selective detection of dopamine (DA). The sensing system is based on hazelnut kohl (the black soot of kohl) which is used as a carbon source. Generally, kohl is a traditional eye cosmetic that used in different parts of the world and synthesized by the combustion process like burning natural materials. Here, it has been proven that black soot (kohl) obtained from hazelnut has a carbon dot structure and can be used for sensory applications. Some characterization methods are carried out to reveal the kohl structure. Also, the photoluminescence properties of the prepared CDs of kohl are investigated. It is found that the size of CDs is 2–4 nm. Besides, under the optimal conditions, the fluorescence of CDs is used for DA determination. CDs fluorescence intensity is decreased linearly with the increase of DA concentration. By using the fluorescence dependency toward the DA concentration, DA can be determined in the range 0.5–30 μM with the limit of detection of 0.30 μM . Finally, this method is successfully applied to discriminate the DA in the real samples (healthy human serum and cerebrospinal fluid (CSF)) which shows acceptable efficiency for diagnostic purposes.

Keywords Dopamine · Hazelnut kohl · Carbon dots · Fluorescence spectroscopy · Sensor

Introduction

Dopamine (DA) is an essential catecholamine neurotransmitters in central nervous systems that has been discovered in mammalian brain in the 1950s [1]. Abnormal DA concentration in the brain may lead to some neurological system diseases like Parkinsonism, schizophrenia, Alzheimer and euphoria [2–4]. Hence, selective and precise measurement of DA is extremely important for clinical diagnosis. In the past few years, various analytical methods have been developed to determine DA effectively including high-performance liquid chromatography [5], electrochemical analysis [6], Electrogenerated chemiluminescence (ECL) [7], colorimetric [8] and fluorescence method [9]. Among these analytical methods, fluorescence spectroscopy utilizing semiconductor quantum dots (QDs) and carbon dots (CDs) have drawn attention for their attractive attributes. They possess some unique properties such as tunable photoluminescence (PL) properties,

low toxicity, eco-friendliness and good biocompatibility [2, 10, 11]. The most attractive and practical feature of CDs is their fluorescence, which gives the high potential in biological labeling and bioimaging applications [12–14]. Additionally, fluorescence spectroscopy has outstanding features such as low cost, practicability, high sensitivity and good selectivity [15].

CDs as carbonaceous nanomaterial were first synthesized by Xu et al. in 2006 during separation and purification of single walled carbon nanotubes by the arc-discharge method [10, 16]. They are recently discovered as nanoparticles which are quasispherical with sizes below 10 nm [17]. Typically, sized below 10 nm, CDs exhibit unique and useful optical features such as high photostability, excitation wavelength-dependent fluorescence and tunable emission color which have not seen in their nanocarbon relatives (graphenes, fullerenes and carbon nanotubes) [18].

CDs can be synthesized using either top-down or bottom-up approaches. They are including arc-discharged soot [17], laser irradiation of carbon nanoparticles [19], combustion and thermal routes [20, 21] and thermal carbonization of molecular precursors [22]. Among all these synthetic methods, combusting organic material is an elegantly simple source of

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producing CDs [17]. Interestingly, through producing lab-made kohl (surmah) from hazelnut by the combustion process, we found that the kohl is containing CDs. Kohl is a traditional eye cosmetic which is widely used in different parts of the world [23]. It's an ophthalmology and ophthalmic product which is known as surmah in Iran, kohl or cohol in Arabic countries, kajal in India and tiro in Nigeria [24, 25]. They are mostly prepared using natural sources such as leaves, nuts (e.g., hazelnut), beef, oils and so on.

Actually, cost and availability of precursor material are the prime concerns for production of CDs. So, a simple and economical manner for synthesizing of CDs has been attracting in recent years [26, 27]. In this study, soot-based hazelnut kohl is synthesized by the combustion process. It is demonstrated that it has a nanoparticle structure and can be used as an affordable source of CDs, which can be used in analytical chemistry. CDs with smaller sizes are synthesized by an easy-done and green method of kohl production, without using any chemical reagents. According to the best of our knowledge, there is no report on developing a facile, low cost and selective fluorescent sensor based on CDs of hazelnut kohl for detection of DA in alkaline solution. As it is discussed before, DA in alkaline environment has a quenching effect on the fluorescence of CDs [28–30]. The presented sensing system for DA is low cost, effortless, environments-friendly and less toxic which shows adequate performance for biological samples.

Experimental

Reagents

All reagents and chemicals used in this work were of analytical grade and utilized without further purification. Amino acids and DA were purchased from sigma. Calcium chloride (CaCl_2), ascorbic acid, cysteine, sodium hydroxide (NaOH), hydrochloric acid (HCl), phosphoric acid (H_3PO_4), acetic acid (CH_3COOH), sodium chloride (NaCl), urea ($\text{OC}(\text{NH}_2)_2$), maltose, ammonium chloride (NH_4Cl), uric acid ($\text{C}_5\text{H}_4\text{H}_4\text{O}_3$), sodium nitrate (NaNO_3), glucose and aniline were obtained from Merck Chemical Company. Boric acid (H_3BO_3) was purchased from BDH laboratory reagents. All solutions were provided by dissolving the reagents in deionized water. Aqueous universal buffer solutions (pH 2.0 to 12.0) were prepared by addition of appropriate amount of 0.2 M NaOH solution to the mixture of boric acid, acetic acid and phosphoric acid (all 0.1 M) [31].

Apparatus

Fluorescence spectra were recorded on a Perkin-Elmer LS50B fluorescence spectrophotometer with excitation slit of 10 nm. The fluorescence cell was water-thermostated at 25 °C using a

PECO thermostat bath (Shiraz, Iran) equipped with a water circulator. The pH was measured using a Metrohm pH-meter model 827. The UV-VIS absorbance spectra were recorded using a BEL photonics, spectrum lab 53 (Italy), equipped with a quartz analytical cells of 1 cm path-length. Fourier transform infrared (FT-IR) spectra were recorded on a Perkin Elmer FT-IR spectrometer—spectrum RX1 equipped with a Perkin Elmer ZnSe ATR sampler accessory. High Resolution Transmission Electron Microscopy (HRTEM) images were taken on a JEOL instrument (JEM – 2100 F at 200 KV).

Synthesis of CDs from Hazelnut Kohl

Kohl is usually prepared by burning natural materials like hazelnut in the air. In a typical burning procedure, a medium sized hazelnut is burned in the air. To collect the soot, a glass plate is put 50 cm apart on the top of the flame. At the end of burning, the soot-based hazelnut kohl is scraped out with spatula and stored in sample tubes.

The soot-based kohl is then dispersed in alkaline solution to separate the CDs: a 0.12 g portion of kohl is added into 5.0 mL NaOH (1 M), and the mixture is sonicated for 30 min. The smaller particles are collected by removing the large particles through centrifugation at 12000 rpm for 30 min. Then, the solution is filtered with a 0.22 μM syringe filter and the resulting solution is kept in refrigerator for sensory applications.

Procedure for Sensing of DA

The typical procedure for detection of DA by kohl-based CDs was carried out as follows: 400.0 μL ($0.002 \frac{\text{mg}}{\text{mL}}$) of CDs solution from its stock solution was diluted to 1.5 mL using universal buffer solution (0.1 M, pH 12.0) in the volumetric flask. Fluorescence data were recorded at 5 min after each addition of the DA (100.0 μL with desired concentrations) at the excitation wavelength of 260 nm, the scan speed of 500 nm min^{-1} and slit widths of 10 nm.

Results and Discussion

Characterization of Hazelnut CDs

High-resolution transmission electron microscopy (HREM) was the first characterization method that is used for kohl characterization. As shown in Fig. 1, hazelnut kohl contained CDs with a frequently microsphere structure. It was found that the spherical particles were dispersible in alkaline solution, and the size of CDs was 2–4 nm. One can find that the inter-layer spacing of CDs is about 0.32 nm, which is close to that of (002) planes of graphite [32].

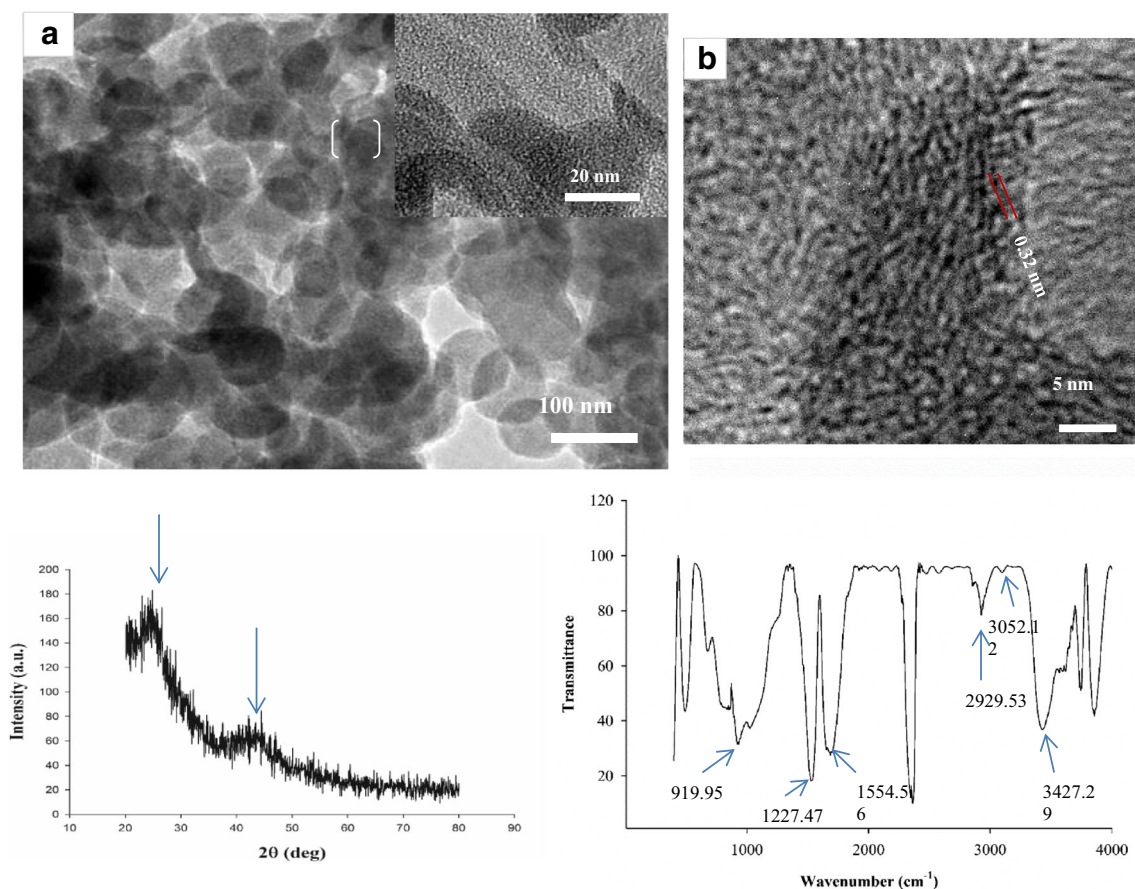


Fig. 1 Characterization of the CDs made from hazelnut kohl and dispersed in NaOH (1.0 M): Upper panel shows the HRTEM images of with 3 different magnifications. Lower panel: (left) XRD spectrum and (right) FT-IR spectrum of the CDs

Lower panel of Fig. 1 displays the powder XRD spectrum of CDs from soot-based kohl. It shows a sharp peak at around $2\theta = 23^\circ$, corresponding to reflections of graphitic carbon, which indicates that the CDs of kohl are crystalline [32]. Another peak at $2\theta = 44^\circ$, indexed to the reflections of the hexagonal graphite crystal structure [33]. The FTIR spectrum of CDs is also shown in lower panel of Fig. 1. The bond around at 3427 cm^{-1} is assigned to the O–H stretching vibration and the band at 2929 cm^{-1} is attributed to C–H stretching deformations. A weak absorption band at 3050 cm^{-1} corresponds to the C=C–H stretching vibration. It is accepted that some unsaturated groups would produce in the burning procedure of the flammable materials [34]. The absorption band at 1554 demonstrates that CDs contain some aromatic structures. The C–O stretching band at 1227 cm^{-1} indicates that CDs have oxygen-containing groups too. The peak at 919 cm^{-1} represents the aromatic hydrocarbons. Generally, the FTIR results showed that CDs are aromatic skeletons in the core and some oxygen-containing groups and alkyl groups at the surface [35].

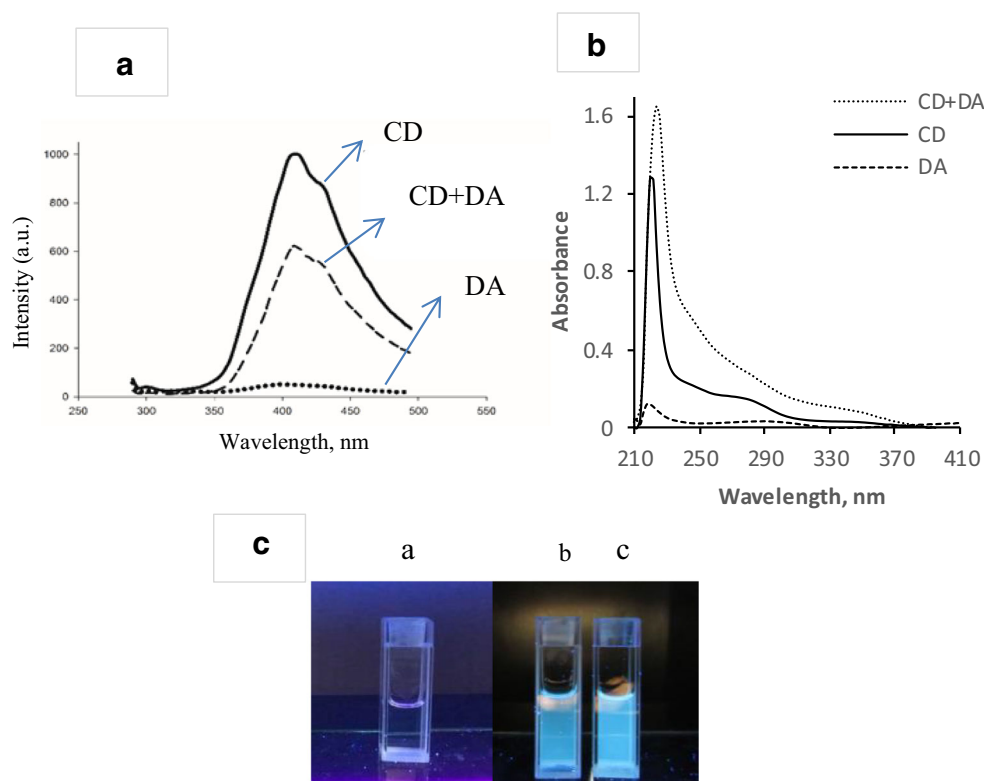
The results of the optical investigations on the CDs are shown in Fig. 2. The colorless CDs solution emits a blue emission under UV irradiation (365 nm) (Fig. 2 (inset C)).

According to the fluorescent spectra of CDs, the maximum excitation and emission wavelength of CDs are observed at 220 and 409 nm, respectively. It is observed from Fig. 2B that absorbance spectrum of CDs is associated with three absorption peaks. The intense peak located at 220 nm gives information about the physical size of the graphite structure [20, 35]. The two others, (one appeared as a shoulder at 270 nm and another as a weak peak at around 340 nm), which demonstrate respectively the π - π^* and n - π^* transitions [36], can be attributed to the presence of C=C, oxygen containing groups and aromatic structures in the hazelnut kohl CDs, as demonstrated by FTIR results. Addition of DA to CDs caused slight changes in the absorbances 270 nm and 340 nm peaks, suggesting interacting of the DA with the conjugated part (π system) of the CDs.

Sensing of DA by Kohl-Based CDs

As shown in Fig. 2A, the fluorescence intensity of CDs is quenched by addition of DA in the wavelength range of 350–500, whereas DA doesn't have significant fluorescence characteristics compared to CDs in this wavelength range. So,

Fig. 2 Photoluminescent (A) and absorbance spectra (B) of the carbon dots in the absence and in the presence of DA 25.0 μ M and (C) the digital photos of DA (a) and CDs in presence (b) and absence (c) of DA under UV irradiation



decreasing in fluorescence intensity of CDs was used as a sensing criterion to detect and measure DA.

Optimization of the Experimental Condition

In the first step, some important parameters such as the amount of CDs, pH of buffer solution, temperature, incubation time and stability of the sensor response were optimized.

To decrease the consumption of the as prepared CDs solutions, the stock solution of CDs was diluted in various ratios, and the fluorescence intensities for each dilution were measured. As shown in Fig. 3, the highest fluorescent intensity was obtained when CDs stock solution was diluted 2, 3 and 4 times with water. Further dilution resulted in decreased fluorescence intensity. To use lower amount of CDs as much as possible and in the same time has the highest fluorescence intensity, fourfold dilution of CDs stock solution was selected as the optimum value for further experiments.

To investigate the effect of pH on the performance of the CDs sensing system, the universal buffer (0.1 M) was selected and the response of the sensor was evaluated in different pH values in the range of 7.0 to 13.0. In acidic pH conditions ($\text{pH} < 7.0$), the organic compounds such as carboxylic groups are more accessible on the surface of CDs. Carboxyl coated CDs absorb some emission signals while hydroxyl would advance fluorescence emission with strong electron donated ability. So, hydroxyls-coated CDs had better fluorescence emission ability than carboxyls-coated [37]. Thus, the

fluorescence quenching in the alkaline solution is more efficient. In these conditions, DA was oxidized to dopamine-quinine [38]. Photoexcited electrons of CDs can transfer to dopamine-quinine and it might be the causes of fluorescence quenching [39]. As shown in Fig. 4a, the CDs represented the highest fluorescence intensity at pHs 11.0 and 12.0. However, the extent of quenching (as shown in Fig. 4b) is largest at pH 12.0. Consequently, next experiments were conducted in pH 12.0.

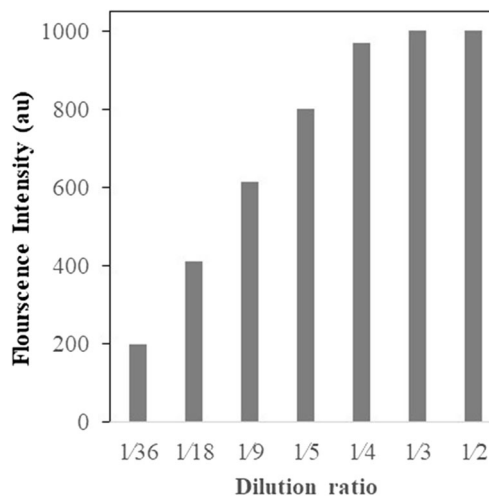


Fig. 3 The effect of dilution on the fluorescence intensity of the as-prepared hazelnut-based CDs: λ_{exc} and λ_{em} were 260 nm and 409 nm

Fig. 4 The effect of pH on the fluorescence intensity of DA, CDs and CDs+DA solutions (left) and on the fluorescence quenching CDs-DA system (right). Universal buffer solutions of 0.1 M and DA of 25.0 μM DA were used

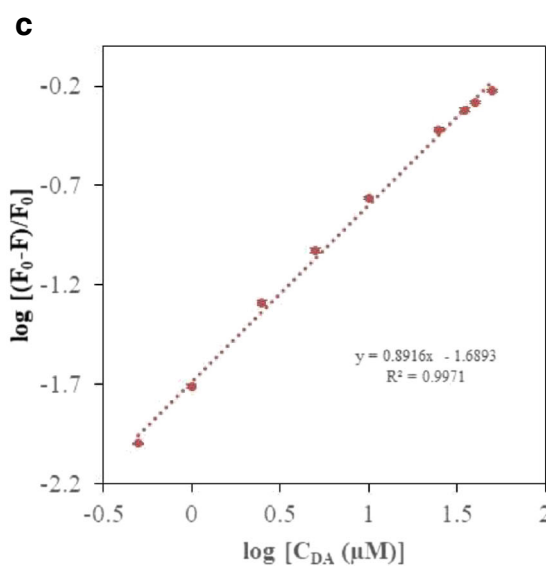
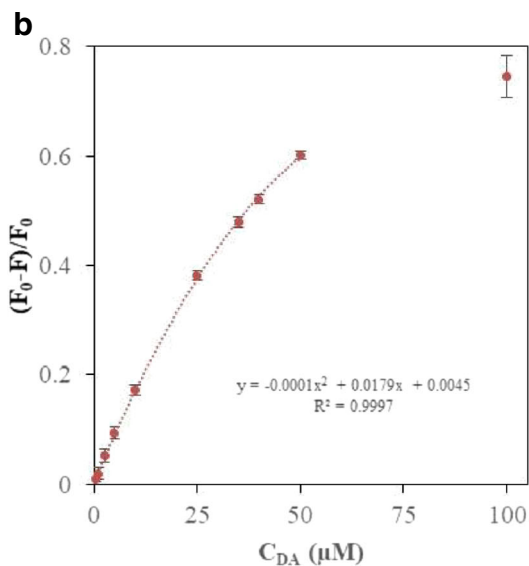
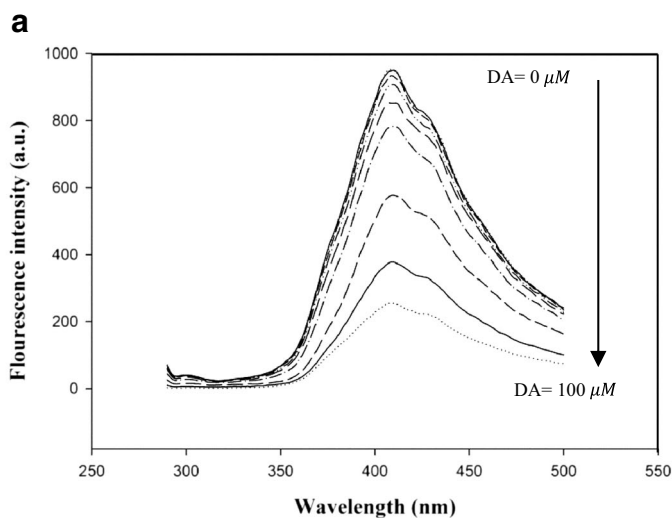
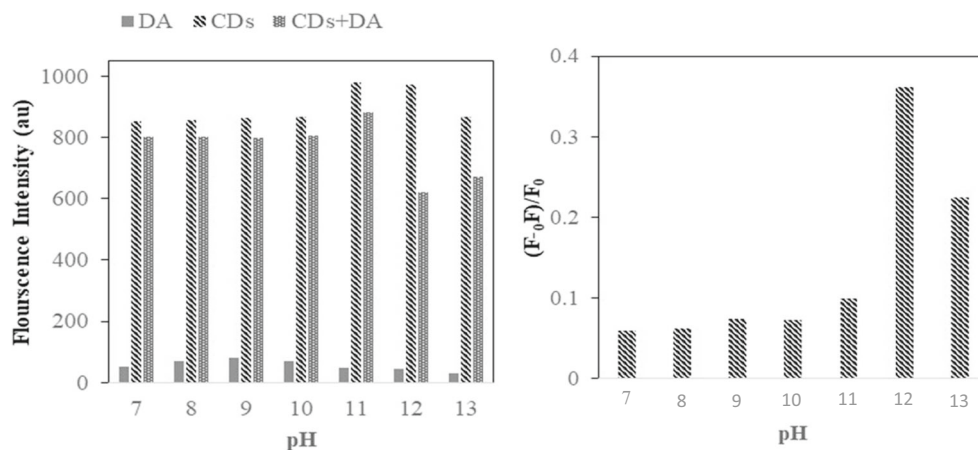


Fig. 5 (A) Changes in the fluorescence spectra (λ_{exc} 260 nm) of the kohl-made CDs in the presence of different amounts of DA (0.5, 1, 2.5, 5, 10, 25, 35, 40, 50 and 100 μM). (B) Relative changes in the fluorescence intensity at 490 nm as function of DA concentration, where the dotted line represents fitting to a quadratic regression model. (C) Double logarithm

transformation of the plot shown in part B (in the concentration range of 0.5–50 μM), where the dotted line represents fitting to a linear regression model. Error bars represent the standard deviation of measurements (RSD \cong 3.0%)

Table 1 The comparison of the proposed method with other reported methods of DA determination

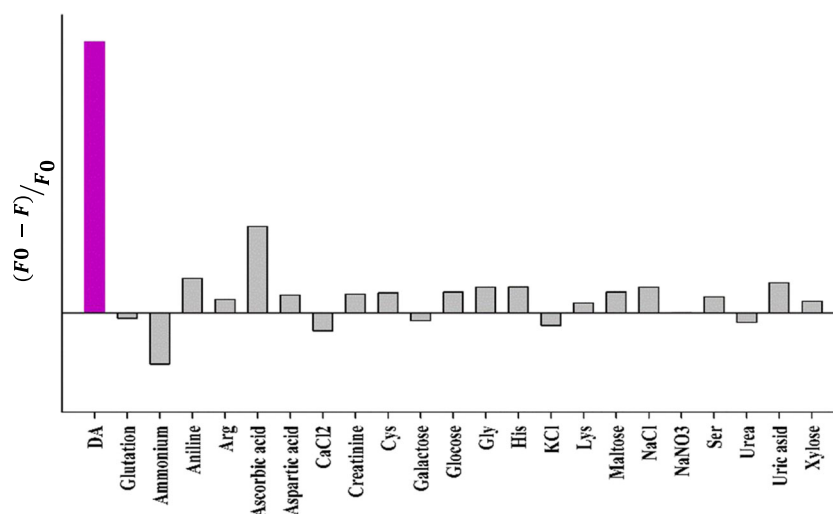
Analytical method	Linear range (μM)	LOD (μM)	Cost
TGA- CdS QDs [42]	1.0–17.5	0.6	cheap
Graphene modified electrode [43]	4.0–100.0	2.6	cheap
Fluorescence of gold nano cluster [44]	–	0.7	Expensive
Aptamer-based colorimetric Biosensor [8]	0.5–5.4	0.3	cheap
DECDs-Au NPs [45]	0.5–3.0	0.2	Expensive
2,3- Diaminophenazine based on Fluorescence quenching [46]	2.0–61.0	1.7	cheap
This work	0.5–50	0.3	cheaper and simpler

Investigating the effect of temperature on the fluorescence quenching CDs after addition of DA (25.0 μM) shows that the performance of the sensing system is not significantly affected by temperature (Fig. S1). So, the ambient temperature was considered as the optimum temperature for subsequent experiments.

The stability of sensor was evaluated by time using two different methods. Firstly, the fluorescence characteristics of CDs in the absence of DA were monitored over time for 2 h in 10 min intervals (Fig. S2). A stable fluorescence was observed. In the second experiment, the fluorescence intensity of CDs after interaction with DA was investigated. This helps to find the optimum reaction time as well. As shown in Fig. S2, 5 min is required for completion of the reaction between CDs and DA. After that, the signal was stable for around 2 h.

Determination of DA

A gradual decrease in the fluorescence intensity of CDs was observed by increasing the concentration of DA (Fig. 5A). The calibration graph was constructed by plotting the relative decrease in fluorescence intensity $(F_0 - F)/F_0$ (where F_0 and F are respectively the fluorescence intensity of the system in the absence and presence of DA) versus the concentration of DA.

Fig. 6 Selectivity of the sensing system: quenching effect of various compounds (equimolar concentration of 25.0 μM) on the fluorescence intensity of CDs

A well-defined quadratic relationship was achieved between the relative fluorescence changes and DA concentration in the concentration range of 0.5–50 μM (Fig. 5B). As shown in part C of Fig. 5, the quadratic relationship can be transformed into linear calibration graph by double logarithm transformation. The limit of detection (LOD) was calculated using the equation suggested by IUPAC as $\text{LOD} = (y_b + 3 s_b)/m$, where y_b , and s_b stand for average and standard deviation of blank signal for 5 repeated measurements, and m denotes the slope of calibration curve [40]. Using this formula, a LOD of 0.30 μM was estimated. The relative standard deviation (RSD) was around 3% for all measurements.

Table 1 compares analytical features of our proposed method with some typical methods used for DA detection. Our method represented comparable and in some instances, better results. A bold advantage that can be encountered for our method is simplicity of preparing the sensing regent.

Selectivity of the Method for DA Detection

To investigate the selective of methods toward DA, the fluorescent signals of CDs in the presence of some foreign substances of possible interferences such as glutathione,

Table 2 Detection results of DA real samples based on the proposed method

Sample	spiked (μM)	Found (μM)	Recovery (%)	RSD (%) ($n=3$)
Human serum	7.0	7.3	104	1.7
	13.0	12.3	95	0.7
	20.0	19.4	97	1.7
Cerebrospinal fluid (CSF)	7.0	7.1	102	1.9
	15.0	13.6	90	1.4
	20.0	18.9	94	1.5

ammonium, aniline, arginine (arg), ascorbic acid, aspartic acid, calcium chloride (CaCl_2), creatinine, cysteine (cys), galactose, glycine (gly), histidine (his), potassium chloride (KCl), lysine (lys), maltose, sodium chloride (NaCl), sodium nitrate (NaNO_3), serine (ser), urea, uric acid and xylose were measured and compared with that of DA at the same concentration ($25.0 \mu\text{M}$). As shown in Fig. 6, only DA caused a dramatic decrease in the fluorescence intensity, while, no or small changes in the fluorescence intensity were observed in the presence of other species. The only possible interfering species is ascorbic acid.

Application to Real Sample Analysis

To determine the practicality of the designed probe, the sensor was used to assay DA in two different matrices (healthy human serum and cerebrospinal fluid (CSF)). The content of DA in plasma of the healthy people is in the range of $0.01\text{--}1 \mu\text{M}$ [41]. However, abnormal ranges higher than $16 \mu\text{M}$ have also been reported [2]. So, the plasma content of DA is in the working range of our method. The DA concentration in CSF is lower than the detection limit of our method. However, we applied our method to determination of DA in CSF as a proof of concept to show the suitability of our method for successful analysis of complex samples.

CSF was taken from 41-year old man who had a stroke from Namazi-hospital Shiraz, and multi-control serum (5.0 mL) was obtained from Ziest Chem Diagnostics [1]. The 100-fold diluted samples were spiked with 7.0, 15.0 and $20.0 \mu\text{M}$ of DA and the concentration of DA in the samples was determined by the standard additions method. As shown in Table 2, satisfactory recoveries of DA in human serum and CSF samples were obtained. Thus, the proposed sensing system may hold potential application for DA detection in biological samples.

Conclusion

The results from the present study demonstrated an effective and selective fluorescence method for DA detection in

alkaline solution. In this procedure, the fluorescent CDs, which are prepared by the combustion process, are used as probe. They were synthesized by burning the hazelnut to obtain black soot that is called kohl. Although the CDs preparation precursor is completely different from previous ones, the obtained CDs had similar HRTM images, sizes and aromatic compounds giving them similar UV-VIS absorption and photo-luminescence features. It should be noted that the proposed sensor based on kohl is simple, rapid and convenient, also all the materials used in the study are low toxic or even non-toxic that avoids environmental pollution. Eventually, the kohl based sensor has the potential for the determination of DA in biological samples with satisfactory results.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10895-020-02668-y>.

Availability of Data and Materials Not applicable.

Authors' Contributions Neda Chavoshi; design the work, acquiring data and preparing first draft.

Bahram Hemmteenejad; Conceptualization, supervision, validation and writing the final draft.

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Compliance with Ethical Standards

Competing Interests There are nothing to declare

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