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A Green and Simple Carbon-dot-based Fluorescent Probe for Selective and Sensitive Detection of Ranitidine Hydrochloride

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Herein, a novel fluorescent probe was designed and synthesized for the selective and sensitive detection of ranitidine hydrochloride based on the quenched fluorescence signal of carbon dots (CDs). The one-step hydrothermal treatment of *Urtica dioica* extract was used to prepare CDs. The as-synthesized CDs exhibited excellent water dispersibility and had a blue color under UV light irradiation (365 nm) with 12.49% of quantum yield (QY). The structural and optical properties of CDs were investigated using UV-Vis spectrophotometer, transmission electron microscopy (TEM), and Fourier transform infrared (FT-IR) spectroscopy. The as-synthesized CDs were used as a simple, sensitive, and inexpensive probe for the detection of ranitidine hydrochloride in pharmaceutical samples. The absorption spectrum of ranitidine overlapped with the excitation spectrum of CDs and the fluorescence intensity of CDs effectively decreased with the increase of ranitidine concentration due to the inner filter effect (IFE). A fluorometric assay was formed based on these findings that had a linear response in the ranitidine hydrochloride concentration range of 0.167-14.03 μg ml^{-1} with a detection limit as low as 0.081 μg ml^{-1}. This new sensing assay was green and had beneficial features such as simplicity, rapidity, inexpensiveness, and ease of operation without the need for further modification. Using the suggested method, ranitidine hydrochloride was successfully measured in the pharmaceutical preparations.

Keywords: Carbon dots, Green synthesis, *Urtica dioica*, Ranitidine hydrochloride, Fluorescence quenching

INTRODUCTION

Ranitidine is a specific potent H_2-receptor antagonist that inhibits gastric acid secretion by blocking acid-producing cells in the stomach. This drug is available in the form of tablets and injections [1]. Ranitidine was mainly used to cure gastroesophageal disease, peptic ulcer disease, and Zollinger-Ellison syndrome. Long-term or excessive consumption of ranitidine leads to some adverse effects including headache, skin rashes, tiredness, constipation, nausea, hypersensitivity, urticarial, *etc.* [2,3]. Recently, the U.S. Food and Drug Administration announced that it is requesting manufacturers to immediately withdraw all ranitidine drugs from the market due to discovering a contaminant known as N-nitrosodimethylamine (NDMA) in ranitidine medications [3]. Different analytical methods such as high-performance liquid chromatography [4], liquid chromatography-tandem mass spectrometry (SDME-LC-MS/MS) [5], electrochemical approaches [6], capillary electrophoresis [7], chemiluminescence [8], and spectrophotometry [9] have been used for the detection of ranitidine. Although these methods are sensitive and useful, they require expensive instruments, professional operators, and costly reagents and are time-consuming. Therefore, providing a simple, inexpensive, and reliable procedure for the detection of ranitidine is necessary.

Recently, fluorescence-based methods have attracted growing attention due to some advantages such as low cost, facile operation, reproducibility, high speed, and cost-effectiveness [10-14]. On the other hand, conventional

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semiconductor quantum dots and fluorescent organic dyes exhibit some limitations such as difficulty in synthesis, chemical instability, and physiological toxicity. The above-mentioned limitations have led to the application and development of carbon quantum dot (CD)-based systems that have been emerging over the last decade [15,16]. CDs are a novel category of zero-dimensional fluorescence nanoparticles with a size of less than 10 nm [17]. CDs are mainly used in drug delivery [18], photocatalysis [19], bioimaging [20], sensing [21,22], light-emitting devices, and photochemical energy storage [23]. In comparison with the semiconductor quantum dot, the applications of CDs are growing owing to their competitive advantages such as less cytotoxicity, stable photoluminescence, facile functionalization, high solubility in water, and excellent dispersibility [24]. Several methods such as microwave digestion [25], ultrasonic oscillation [26], hydrothermal synthesis [12], and electrochemical process [27] have been reported for synthesizing CDs [28]. Among these methods, hydrothermal synthesis is more attractive because it is simple, economical, and highly efficient, and uses natural materials as CD precursors [29].

Inner filter effect (IFE) is one of the fluorescence quenching mechanisms in which the absorption spectra of the quencher overlap complementary with the excitation or emission spectra of the fluorophore. There are two types of IFEs: (i) primary IFE (pIFE) and (ii) secondary IFE (sIFE). When the absorption spectra of a quencher overlap with the excitation wavelength of a fluorophore, it is called pIFE, and if the absorption spectra of the quencher overlap with the emission spectra of the fluorophore, it is called sIFE. While the absorption spectra of the fluorophore would not change in the presence of the quencher, this indicates that no new complex was formed [30-32].

Herein, the authors reported a simple, inexpensive, and green method for the preparation of CDs by one-step hydrothermal synthesis *via Urtica dioica* for the first time. The as-synthesized CDs were soluble in water and exhibited high quantum yield. We found that the fluorescence intensity of the CDs could quench in the presence of ranitidine based on the IFE. To evaluate the applicability of the proposed methods, they were applied for the detection of ranitidine in commercial pharmaceutical products and the result was satisfying.

EXPERIMENTAL

Materials and Instruments

All the chemicals and reagents were of analytical grade and were used without further purification. The standards of ranitidine hydrochloride were supplied by Chemidrou industrial company, Tehran, Iran. L-cysteine, ascorbic acid, glucose, and sodium chloride (NaCl) were purchased from Sigma-Aldrich chemical company. The rest of the chemicals were bought from Merck (Darmstadt, Germany). All the solutions used in this study were prepared using double-distilled deionized water.

The UV-Vis absorption spectra and fluorescence spectra of the CDs were recorded using a Biochrom Biowave II spectrophotometer and an FP-6500 Jasco spectrofluorometer, respectively. The morphology and size of the CDs were surveyed by transmission electron microscopy (HRTEM, JEOL 2010F) at 200 kV. The Fourier transform infrared (FT-IR) spectra were assessed in the form of KBr pellets by a NEXUS 670 FT-IR spectrometer.

Synthesis of CD

The hydrothermal treatment of *Urtica dioica* extract was used for synthesizing CD. Briefly, 100 g of *Urtica dioica* was washed thoroughly in deionized water and dried in dark at room temperature. The dried *Urtica dioica* was crushed and ground to make a powder using an electric hand blender. About 5 g of the as-prepared powder was mixed with 100 ml of deionized water and was heated at 60 ºC for 5 min. Then the solution was filtered to obtain a clear solution, and 0.5 ml of ammonia solution (35%) was added to 15 ml of the filtered solution. The obtained solution was mixed for 10 min to form a homogeneous solution. Afterward, the resultant solution was transferred into a Teflon-lined autoclave and was heated at 170 ºC for 5 h. To obtain pure and highly fluorescent CDs, the solution was centrifuged at 10000 rpm for 15 min. The brown supernatant was collected and was freeze-dried to form a solid powder of nanoparticles. Finally, a 1 mg m^{l-1} solution of CDs was prepared by dissolving the resultant solid powder in deionized water and kept at 4 ºC for further characterization studies and other experiments.

Stock Standard Solution of Ranitidine Hydrochloride (1000 µg ml^{-1})

The stock standard solution of ranitidine hydrochloride was prepared as follows: 0.1 g of the standard sample of ranitidine hydrochloride was accurately weighed and poured into a 100-ml volumetric container. About 30 ml of deionized water was added to the volumetric container and mixed for 15 min to obtain a clear solution. The mixture was diluted to mark with deionized water.

Quantum Yield (QY) Measurement

The QY of the CDs was calculated by comparing the integrated photoluminescence intensity (excited at 310 nm) and absorbency values (less than 0.1/310 nm) with that of the reference solution. The reference was prepared by dissolving quinine sulfate in 0.1 M H_2SO_4 with QY= 54% and η = 1.33, where η is the reflective index of the solvent. Using the slope method, the QY value was calculated as follows:

$$\Phi_X = \Phi_{ST} (m_X/m_{ST}) (\eta^2_X/\eta^2_{ST})$$

where Φ is the QY and m is the slope extracted from the integrated fluorescence intensity *versus* absorbance diagram, respectively. Subscripts ST and X refer to the standard and the sample, respectively [33].

Fluorescence Sensing of Ranitidine

About 50 µl of CD aqueous solution with a concentration of 1 mg ml^{-1} was added to a 5-ml volumetric container with 1 ml phosphate solution as a buffer (100 mM, pH 7). Afterward, various concentrations of ranitidine were added to the above solution and were diluted to 5 ml with deionized water. After 4 min of incubation time, the fluorescence spectra of the solution were recorded in the range of 330-550 nm under excitation at 310 nm.

Sample Preparation

The applicability of the proposed method was evaluated by analyzing tablet and injection samples as real pharmaceutical samples. Ten Raniflax®tablets (containing 150 mg ranitidine hydrochloride) were accurately weighed and thoroughly ground to a fine powder. An accurately weighed portion of the powder equivalent to 150 mg of ranitidine hydrochloride was transferred into a 100-ml volumetric flask. About 25 ml of a degassed mixture of methanol and 0.1 M aqueous ammonium acetate (21:4) were added to the above solution. The mixture was sonicated for 15 min to provide complete dissolution and then diluted to the desired volume with deionized water. Afterward, the produced solution was stirred for 30 min and filtered through a filter paper. In the end, the filtered solution was pretreated and analyzed according to a procedure similar to the one mentioned above.

To prepare the injection samples, five ampoules (label value of each injection: 25 mg ml^{-1}) were mixed and sonicated for 15 min. Then a certain amount of this mixture was diluted with deionized water to obtain solutions with different concentrations which were analyzed by the above procedure.

RESULTS AND DISCUSSION

Characterization of CDs

Fluorescence and UV-Vis spectroscopy were applied in order to describe the optical properties of CDs and the obtained results are illustrated in Fig. 1. The peaks at 223 nm and 262 nm in the UV-Vis spectrum were ascribed to the π-π^* transmission of the C=C band [34,35]. According to Fig. 1, it is readily apparent that under excitation at 310 nm, CDs displayed a strong photoluminescence (PL) emission peak centered at 398 nm, and had a blue color under UV light radiance (inset of Fig. 1). To examine the excitation wavelength dependence of fluorescence spectra, the fluorescence spectra of CDs were investigated in the range of 260 to 310 nm. As illustrated in Fig. 2, the fluorescence of CDs was dependent on the excitation wavelength. By changing the excitation wavelength from 260 to 360 nm, the emission spectra of CDs shifted to longer wavelengths (redshift) [36]. The fluorescence intensity of CDs reached the maximum amount at the excitation wavelength of 310 nm (Fig. 2). This phenomenon is one of the intrinsic properties of CDs that could be due to different factors, including energy traps and functional groups [37]. There are plenty of functional groups such as carboxylic and amino groups on the surface

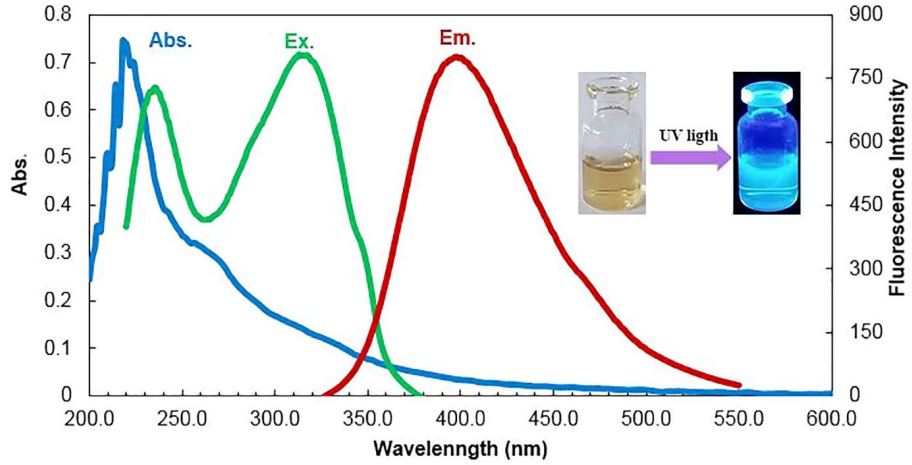

Fig.1. UV-Vis spectrum (blue line), fluorescence excitation (green line), and emission (red line) spectra of CDs. The inset reveals the photograph of CDs under visible and UV light irradiation.

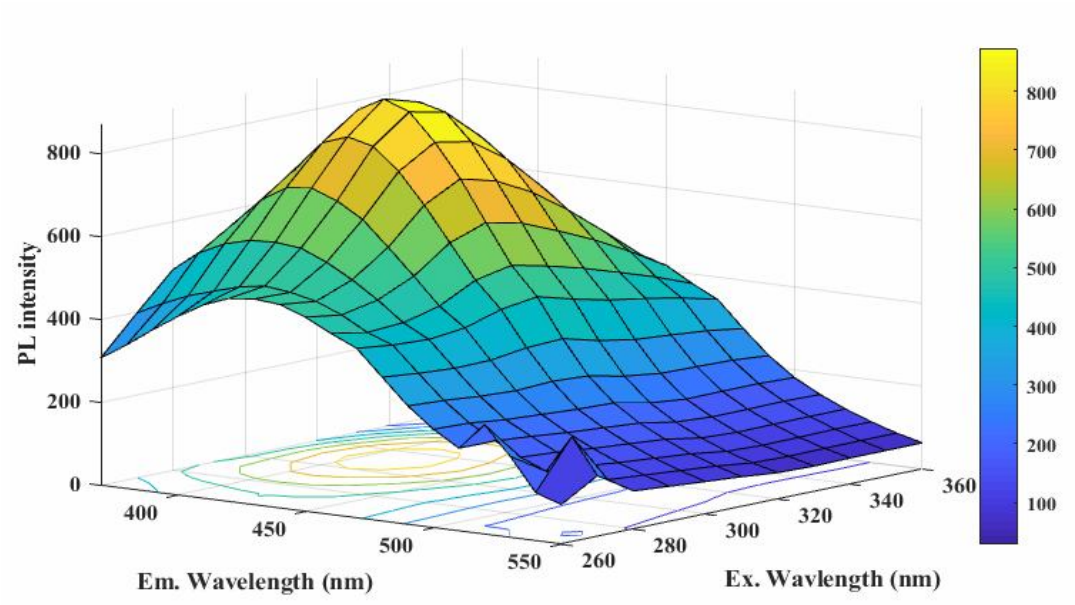

Fig. 2. 3D schematic of emission spectra upon variation of the excitation wavelength from 260 to 360 nm).

of CDs that can lead to the defect sites on the surface of CDs which cause fluorescence emission through the radiative recombination of the excited trapped [12,38-42].

TEM was used for the characterization of the morphology and size of CDs. As shown in Fig. 3, CDs were monodispersed and quasi-spherical in shape. The particle size distribution graph indicates that the size of CDs was in the range of 1 to 5 nm with an average diameter of about 3 (inset of Fig. 3).

The FT-IR spectroscopy technique was used for identifying the functional groups. As shown in Fig. 4, the bands at 3420.39 cm^{-1} and 3256.18 cm^{-1} were ascribed to the stretching vibration of –OH and N–H groups [43,44]. Absorption bands at 2950.49 and 1373.47 belonged to the

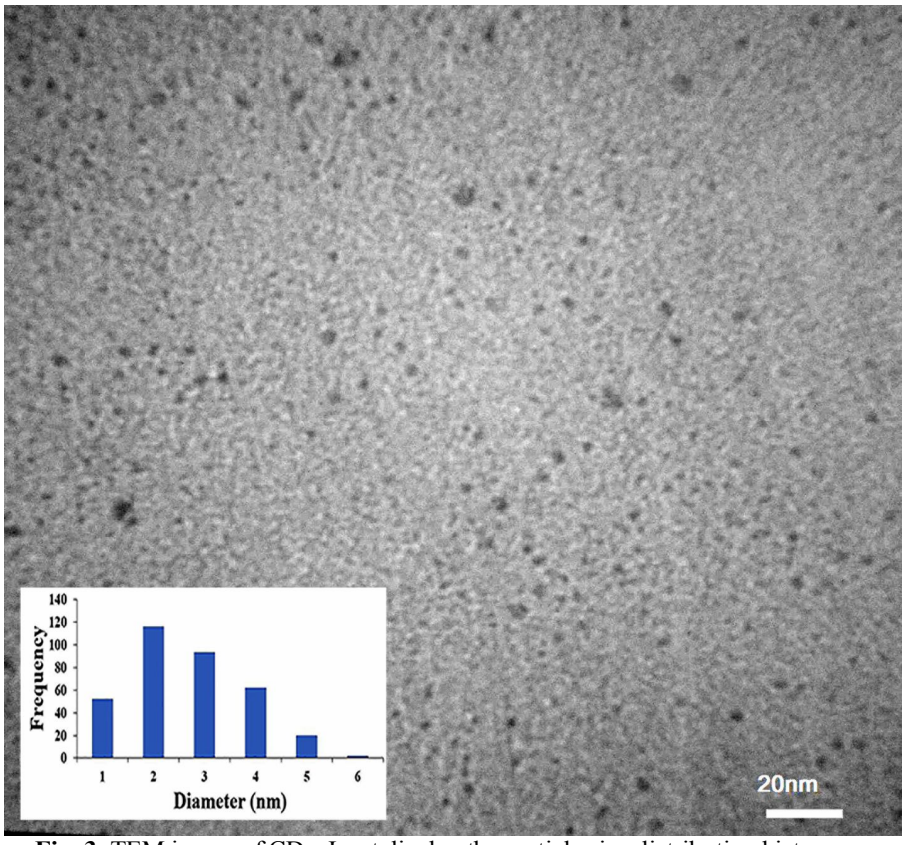

Fig. 3. TEM image of CDs. Inset display the particle size distribution histogram.

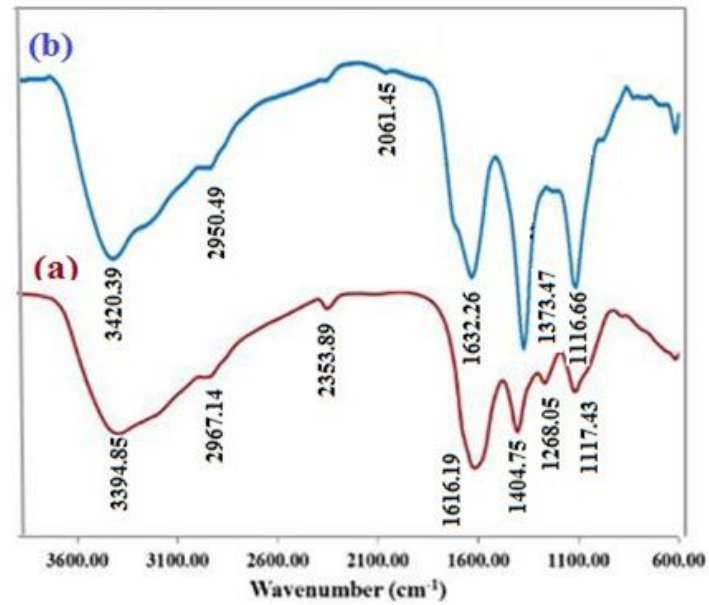

Fig. 4. Fourier transform infrared (FT-IR) spectra of Urtica dioica extract (a) and CDs (b).

stretching vibration of C–H, and the peaks at 1632.26 cm^{-1} and 1116.66 cm^{-1} were attributed to the stretching vibration of C=O and C–N groups, respectively [45,46]. These results indicated that the surface of CDs should be occupied with carboxylic and hydroxyl groups, which led to their high solubility in water [44,47].

Analytical Performance of the CD Probe for Ranitidine

The influence of different factors including pH, buffer concentration, CD amount, excitation wavelength, and incubation time on the detection of ranitidine was investigated upon adding 5 µg ml^{-1} ranitidine. As presented in Fig. 5a, the relative fluorescence intensity of CDs (F_0/F) had no obvious change in the absence and presence of ranitidine by altering the pH from 3 to 12; therefore, pH 7 was chosen. F_0 is the fluorescence intensity of CDs in the absence of ranitidine and F is the fluorescence intensity of CDs in the presence of ranitidine. In Fig. 5b, the effect of the concentration of buffer on F_0/F is illustrated. With a buffer concentration of 10 mM, F_0/F reached the maximum amount; therefore, this concentration was chosen as the optimum concentration of buffer. The influence of different amounts of CDs (1 mg ml^{-1}) on F_0/F was also investigated. As presented in Fig. 5c, F_0/F increased by increasing the amount of CDs from 10 to 50 µl and reached the maximum value at 50 µl and then decreased at higher amounts of CDs. The interaction time is one of the critical factors in the analysis, hence the relative fluorescence intensity (F_0/F) was studied for 6 min after adding 5 µg ml^{-1} ranitidine. As shown in Fig. 5d, the amount of F_0/F remained unchanged after 4 min, so the reaction time was chosen to be 4 min.

The fluorescence intensity of CDs was examined in a solution with different ionic strengths. As shown in Fig. 6, there was a negligible change in the fluorescence intensity upon the addition of NaCl at various concentrations. This indicated that the variation of the ionic strength did not affect the fluorescence intensity of CDs in the detection system.

To evaluate the analytical performance, different concentrations of the stock standard solution of ranitidine were added to CDs in optimized conditions. As indicated in Fig. 7, by increasing the concentration of ranitidine the fluorescence intensity of CDs decreased. Also, according to Fig. 7, there was a linear relationship between the response factor (F_0/F) and the concentration of ranitidine from 0.17 to 14.03 µg ml^{-1} that fit with the Stern-Volmer equation with the correlation coefficient $R^2 = 0.9936$.

$$F_0/F = 1 + K_{SV}[Q]$$

where F_0 and F are the fluorescence intensity of CDs in the absence and presence of ranitidine, respectively. K_{SV} is the Stern-Volmer constant, and Q is the concentration of ranitidine [13]. The limit of detection was calculated to be 0.08 µg ml^{-1} by 3S/m. The proposed method was compared with different methods for the determination of ranitidine, and the results are shown in Table 1.

Possible Mechanism of the CD-based Probe for Ranitidine

The fluorescence quenching mechanism for the detection of ranitidine was studied. As shown in Fig. 1, the as-prepared CDs had two excitation peaks around 235 and 310 nm, and ranitidine exhibited two absorption peaks around 230 and 315 nm (Fig. 8). Thus, a spectral overlap could be accomplished between the absorption spectrum of ranitidine and the excitation spectrum of CDs. These spectral overlaps could lead to high IFE between CDs and ranitidine, resulting in the quenching of fluorescence intensity of CDs [30,31]. In addition, the absorption spectra of ranitidine and the absorption spectra of CDs in the absence and presence of ranitidine were also recorded. As shown in Fig. 8, by adding ranitidine the absorption peaks of CDs did not change. This stability showed that the possible mechanism was not due to the static or dynamic quenching process and could be through the IFE [48]. It also showed that no new complex or substance has been formed, which was another evidence for IFE [32,13].

Based on the experimental results, the authors proposed that the hydrogen bonding between –OH, $–NH_2$, and –COOH at the surface of CDs and ranitidine could be the reason for the interactions of CDs and ranitidine. Also, the tautomeric (enamine and nitronic acid) forms of ranitidine could accelerate the electrostatic interaction and hydrogen bonding between ranitidine and CDs. These specific interactions could increase the IFE and help the as-prepared CDs serve as an efficient and specific chemosensor for the

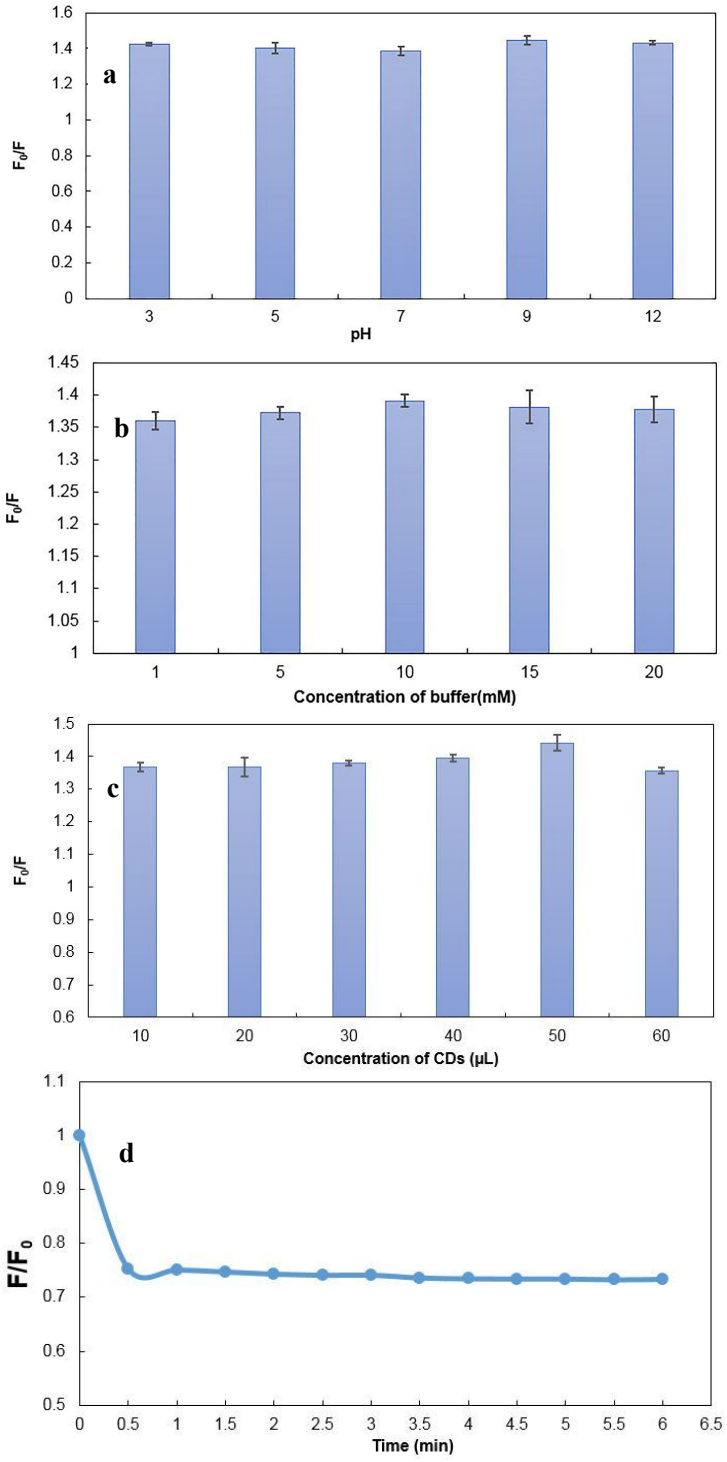

Fig. 5. Fluorescence intensity *versus* different pH values at excitation about 310 nm (a), The effect of concentration of buffer on the response of the sensor (b), fluorescence intensity *versus* different the concentration of CDs (c), the fluorescence intensity of CDs dispersion in water *versus* time after adding of 5 µg ml^{-1} of ranitidine (d).

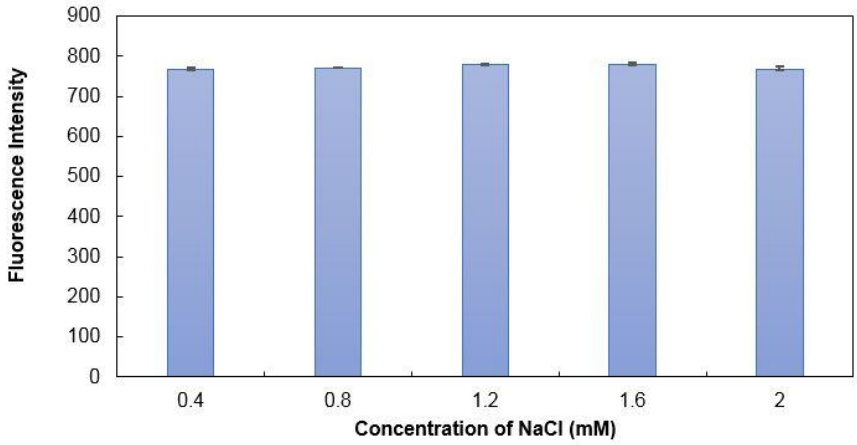

Fig. 6. The effect of NaCl concentration on CDs fluorescence. Error bars represent the standard deviation for three independent measurements.

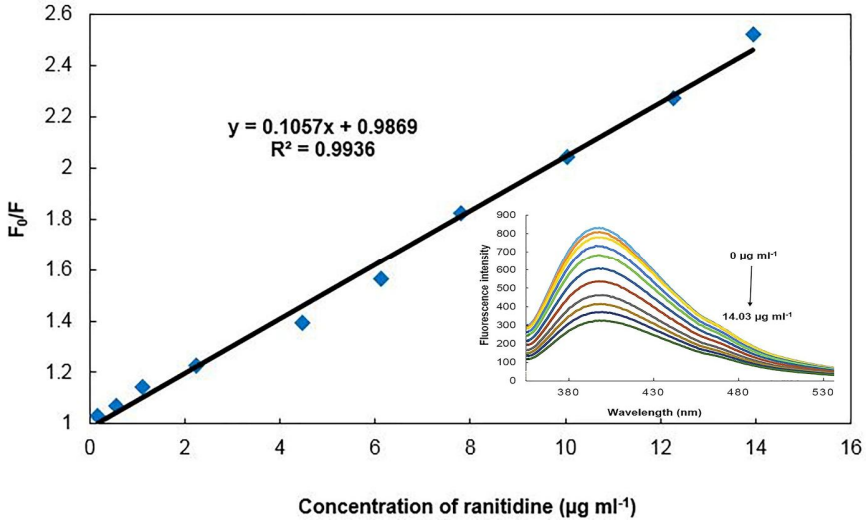

Fig. 7. Calibration plot of the quenching of the CDs *versus* ranitidine, inset: Photoluminescent response of CDs upon addition of various concentrations of ranitidine.

detection of ranitidine. The discussed mechanism for the detection of ranitidine is shown in Fig. 9.

Selectivity, Repeatability, and Analysis of Pharmaceutical Samples

In order to evaluate the selectivity of the nanosensor, the effect of different potential interferences on the detection of 5 µg ml^{-1} ranitidine was investigated. Examined potential interferences included Na^{+}, k^{+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, urea, glucose, cysteine, ascorbic acid, metformin, and famotidine at a concentration of 40 µg ml^{-1}. As illustrated in Table 2, the effect of potential interferences on the sensing system was negligible. The results of these experiments confirmed that the prepared probe has a high specificity for the recognition of ranitidine.

The repeatability of the prepared sensor was tested by

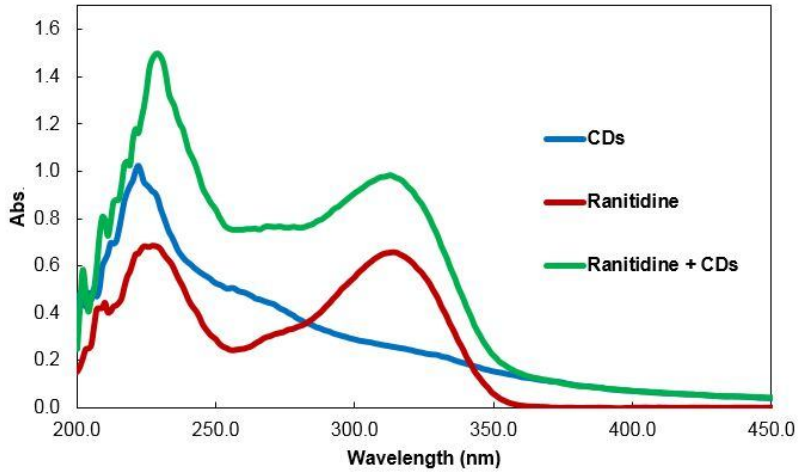

Fig. 8. Comparison of the absorbance of CDs and ranitidine.

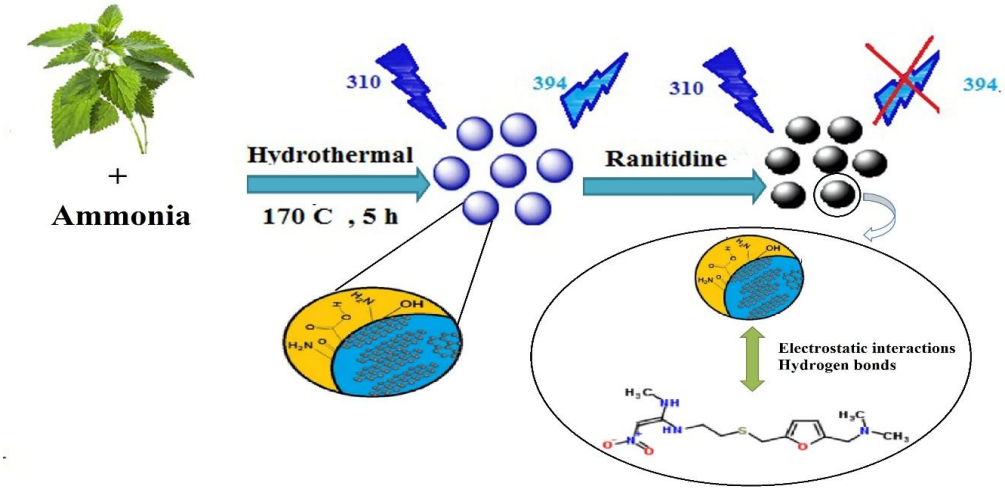

Fig. 9. The possible mechanism for the detection of ranitidine by the as-prepared CDs.

analyzing the standard solutions of ranitidine with a concentration of 8 μg ml^{-1} at various times (n = 6) within 24 h. The relative standard deviation (RSD%) was calculated to be 3.3. To examine the feasibility of this method for the analysis of ranitidine in pharmaceutical products, the commercial products of tablet and injection were analyzed by the standard addition method, and the recovery was examined. The results are summarized in Table 3. It can be seen that the excipients in real samples did not interfere with the detection, and the results were in good agreement with the label claim. Thus, it is clear that the current method can be successfully applied for the detection of ranitidine in real pharmaceutical samples.

CONCLUSIONS

In summary, the authors successfully synthesized CDs *via* a simple and one-step hydrothermal treatment of *Urtica*

Table 1. Comparison Results of the Proposed Method with some Analytical Method for Determination of Ranitidine

Method	Linear range ($\mu g\ ml^{-1}$)	LOD ($\mu g\ ml^{-1}$)	Remarks	Ref.
SDME-CL-MS/MS	0.0098-5.2600	0.0033	Complicated and time consuming	[5]
Electrochemical	0.695-10.105	0.086	Less sensitive	[6]
Conductometric measurement	-	350.87 in water 175.44 in 75:25 hydroalcoholic mixture	Less sensitive	[49]
Capillary electrophoresis	0.5-50	0.088	Less sensitive	[7]
Spectrophotometric	2-16	0.22	Less sensitive	[9]
HPLC	2.50-12.50	0.091	Complicated, less sensitive,	[4]
Chemiluminescence Luminol-H_2O_2-(N,S-CQDs)	0.5-50	0.12	Less sensitive	[8]
Spectrofluorometric Flow injection	0.02-0.5	0.013	Involves flow injection	[50]
Spectrofluorometric CdS QDs	0.05-15.0	0.38	Less sensitive	[51]
Spectrofluorometric NBD-RAN derivative	0.04-1.2	0.04×10^{-3}	Involve extraction, time consuming	[1]
Spectrofluorometric CDs	0.167-14.03	0.081	Simple, sensitive, fast	This work

Table 2. Effect of Different Interferences Species (Concentration of Ranitidine is $5\mu g\ ml^{-1}$)

Species added ($40\ \mu g\ ml^{-1}$)	Change in fluorescence intensity (%)
Na^+	1.75
K^+	1.67
Ca^{2+}	2.01
Mg^{2+}	1.70
Zn^{2+}	2.83
Glucose	3.55
Metformin	-2.92
Famotidine	3.76
Ascorbic acid	0.09
Cysteine	-1.43
Urea	-5.65

Table 3. Determination of Ranitidine in Pharmaceutical by the Method

Sample	Manufacturer	Sample content (μg ml^{-1})	Added (μg ml^{-1})	Found (μg ml^{-1})	Recovery (%)	RSD (%)
Tablet	Chemidarou Tehran-Iran	2	0.4	2.5	104.1	3.2
			4	5.7	95	3.4
			11	13.7	105.4	2.5
Injection	Alborzdarou Iran	2	1	3.2	106.6	3.4
			3	4.8	96	3.5
			10	11.8	98.3	3.1

dioica for the first time. The emission of CDs could be quenched by ranitidine hydrochloride based on the IFE. The method could be successfully applied for the fast and selective detection of ranitidine hydrochloride in the linear range from 0.17-14.03 μg ml^{-1} with the detection limit of 0.08 μg ml^{-1}. In addition, the results obtained from the analysis of real pharmaceutical samples proved the promising potential application of these probes.

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