Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

Available online at www.jcbsc.org

Section A: Chemical Sciences

CODEN (USA): JCBPAT Research Article

Sensitive and Selective Determination of Metronidazole Using Highly Luminescent Pepper Carbon Dots

Naser Samadi*and Saeedeh Narimani

Department of Chemistry, Faculty of Science, Urmia University, Urmia, Iran

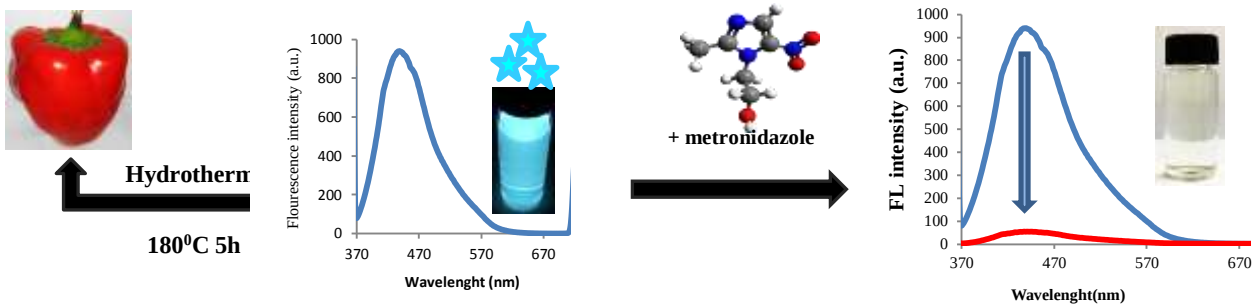
Received: 17 January 2016; **Revised:** 26 January 2016; **Accepted:** 04 February 2016

Abstract: In this work High quality pepper carbon dot fluorescent sensor for determination of metronidazole is reported. The carbon nanoparticles were structurally and optically characterized by UV–vis absorption spectroscopy, photoluminescence (PL) emission spectroscopy and transmission electron microscopy (TEM).

The calibrated curve was linear from 5×10^{-9} molL^{-1} to 7.5×10^{-3} molL^{-1}for metronidazole. The detection limit was calculated as 2.9×10^{-9} molL^{-1}. When adding other drugs and acide amines to the pepper carbon dot solution, fluorescence spectra of pepper did not change significantly but it had good selectivity toward metronidazole. The method presented here is simple, inexpensive, rapid, sensitive and an appropriate method for practical application.

Keywords: Carbon dots; Fluorescence probe; Metronidazole.

Graphical abstract:



INTRODUCTION

Recently, carbon dots (CDs), emerging as a new class of fluorescent nanomaterials, have attracted increasing interests. CDs are small oxygenous carbon nanoparticles with sizes blow 10 nm, displaying size and excitation wavelength dependent photoluminescence (PL) behavior[1].Compared with fluorescent chalcogenide semiconductor nanocrystals (QDs), the CD emitters are promising alternatives due to their excellent optical properties, biocompatibility[2], high photo- stability[2-6], and ease of preparation[7-8] , and low environmental hazard Fluorescent carbon dots (CDs), Thereby,CDs have been considered as a promising choice in potential applications for drug determination[9] biosensing[10], catalysis[11], and imaging[12-14].

During past decades drugs and their determination have been widely studied[15-17] .Metronidazole (MNZ) (**Fig.1**), is a commonly used nitroimidazole antibiotic to treat parasitic putrefactions in human being, including Giardia putrefactions, amebic liver abscess, bacterial vaginosis[18, 19], etc. It is also applied as veterinary medicine to prevent and treat putrefactions and as growth-promoting feed additive in aquaculture industry. Some toxic reactions will be caused, when the accumulated dose of MNZ exceeds a certain value in human bing, for instance, seizures, peripheral neuropathy and ataxia[20].

Therefore, accurate and reliable determination of rare MNZ in biological samples is of great importance for the assurance of consumers' healt. A variety of quantitative analytical methods have been reported to determination of MNZ in different matrices ,including gas chromatography[21] high performance liquid chromatography (HPLC)[22], thin layer chromatography[23] ultraviolet spectrophotometry[24], supercritical fluid chromatography[25], and electrochemical sensor[26-37].But, these methods do not offer acceptable sensitivity and stability and suffer from expensive instrumentation and need complex. For that reason, we are interested to develop a simple, rapid, selective and sensitive method like spectrofluorimetric measurement for simple determination of metronidazole, in which the quenching properties of QDs were used in sensing of metronidazole with high sensitivity and good selectivity.

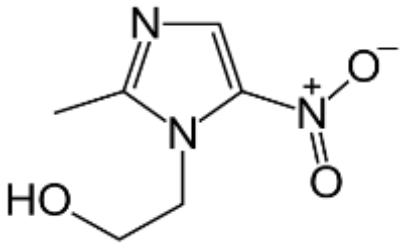


Fig.1. The chemical structure of metronidazole.

EXPERIMENTAL

2.1. Reagents: The pepper was purchased from local supermarket and washed with deionized water for further use.All used materials were of analytical grade. The metal salts Cd $(NO_3).4H_2O$, $Mg(NO_3)_2.6H_2O$, $Ca(NO_3)_2.4H_2O$, $Fe(NO_3)_3.9H_2O$, $BaCl_2.2H_2O$, $MnCl_2.4H_2O$, $Cu(NO_3)_2.3H_2O$, $HgCl_2$, $Pb(NO_3)_2$, $Zn(NO_3)_2.4H_2O$, $Cr(NO_3)_3.9H_2O$, $AgNO_3$, purchased from Merck (Germany). Metronidazole was purchased from Sigma–Aldrich (Germany). A $0.01 molL^{-1}$ phosphate buffer solution (PBS) was prepared using KH_2PO_4 and K_2HPO_4 and used as the medium for QD solutions. All solutions were prepared using doubly distilled deionized water (DDW).

2.2. Apparatus: Fluorescence spectra were measured on a JASCO- FP-6500PC spectrofluorimeter. UV–vis absorption spectra were recorded by a computerized WPA- Biowave II instrument using a l0 mm quartz cell. The pH was measured with a Metrohm 827-pH lab pH-meter. The structure and morphology of QDs were investigated by high resolution transmission electron microscopy (HRTEM) using a Philips-CM 30 model under the accelerating voltage of 100 kv.

2.3. Synthesis of fluorescent C-dots: were prepared by using a simple and rapid procedure according to the reference[37] with some changes. 10g of fresh pepper and 2.5 mL of H_2O were added into a Teflon-lined autoclave and heated at 180 °C for 5 h. The C-dots were collected by removing the large nanoparticles through centrifugation at 8000 rpm for 15 min and finally the obtained product was dispersed in ultrapure water then dialyzed with dialysis bag for 20 h, and the dialysate containing C-dots was disperse in deionized water for further used.

2.4. Determination of mtronidazole by pepper CDs: The fluorescence quenching of pepper CDs by metronidazole was performed in the phosphate buffer solution at pH=7. 0.32 mg of pepper CDs, 1.0mL of PBS (pH=7.0),1.0 ml dionised water and certain amount of metronidazole were sequentially added to a 3mL calibrated test tube. The fluorescence intensity was measured at λem/λex= 435/290 nm. The fluorescence intensity of pepper CDs was assigned as F0. The fluorescence intensity after adding MNZ was assigned as F. The different intensity ($F-F_0$) was plotted versus the concentration of MNZ to obtain a calibration curve.

RESULTS AND DISCUSSION

3.1. Characterization of the pepper carbon dots: Absorption and emission spectra of pepper carbon dots are shown. The wide absorption spectra can be observed in the range of ultraviolet wavelength to around 290 nm. Inset: the photograph of C-dots dispersion under UV light (300 nm) (**Fig.2**). The corresponding emission spectra which were excited at 290 nm showed the maximum fluorescence intensity at 435 nm (**Fig.3**). In addition, the size and shape of the C-dots were characterized by HRTEM

as shown in (**Fig.4**). From the TEM image, it was clearly seen that the C-dots used in this work is regular spherical nanocrystals with semidiameters ranging from 3 to 5 nm.

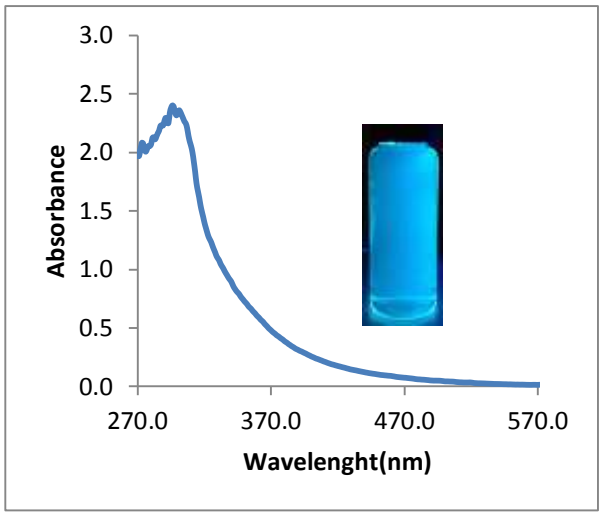


Fig.2. The absorption spectra of pepper C-dots; Inset: the photograph of C-dots dispersion under UV light (300 nm)

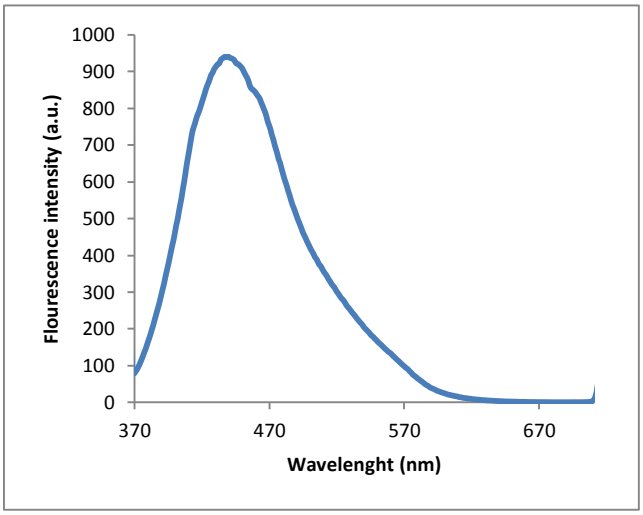


Fig.3. The fluorescence spectra of pepper C-dots.

3.2. Effect of stability: Due to the importance of CDs' stability the stability of pepper carbon dots in different times was studied from (**Fig.5**) it can be seen that after 50 minuts fluorescence spectra of pepper carbon dots did not change significantly that shows C-dots high stability.

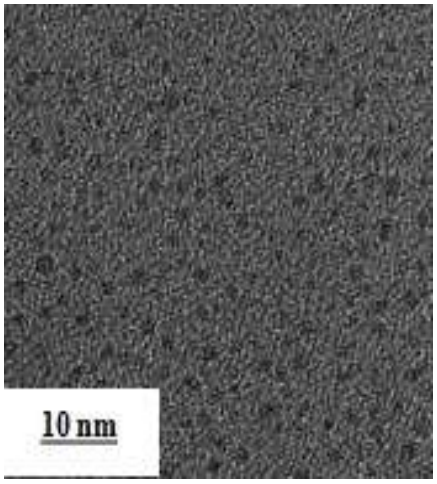


Fig.4. TEM image of pepper carbon dots.

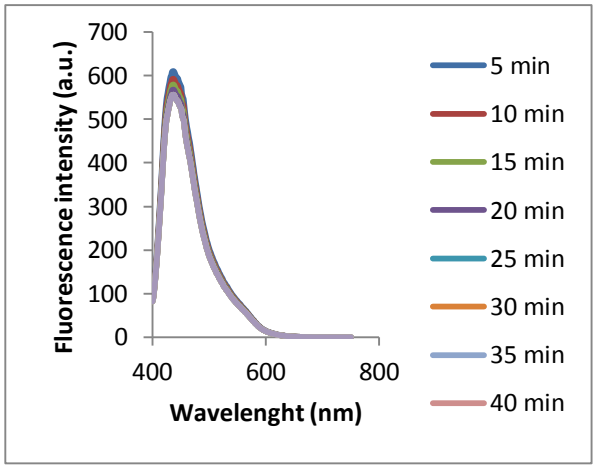


Fig.5. The effect of time on fluorescence spectra of pepper carbon dots.

3.3. Influence of pH on luminescence of pepper carbon dos: The effect of pH from 5 to 11 was studied in order to select the optimum conditions for the determination of metronidazole with the pepper carbon dots by using phosphate buffers. From (**Fig.6**) it can be seen that pH influences the fluorescence intensity of QDs in the absence and presence of metronadazole. It can be seen that pH=7 affected the interaction between C-dots and metronidazole more than other pHs. Thus, the optimal pH=7 was selected in this study.

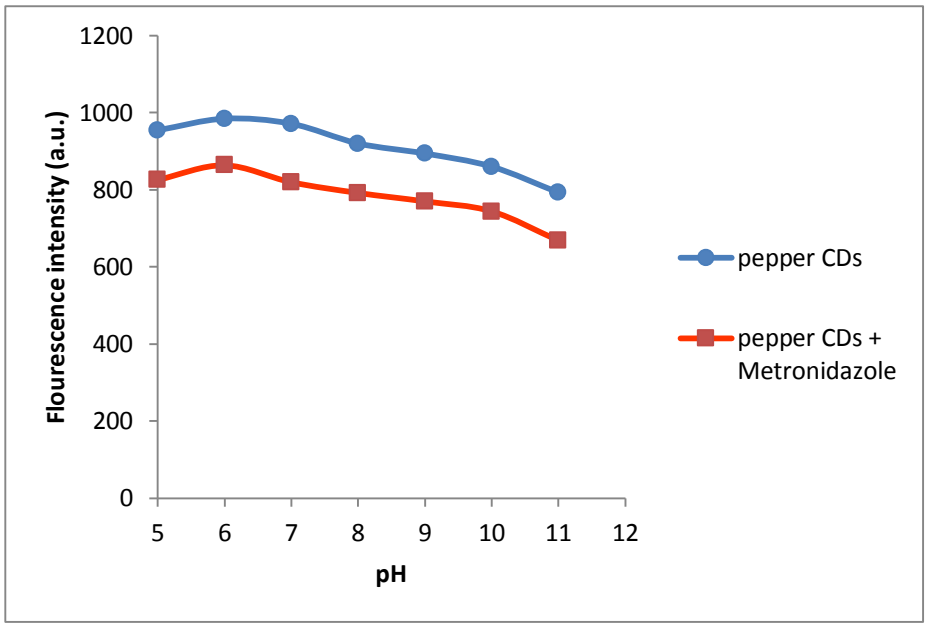
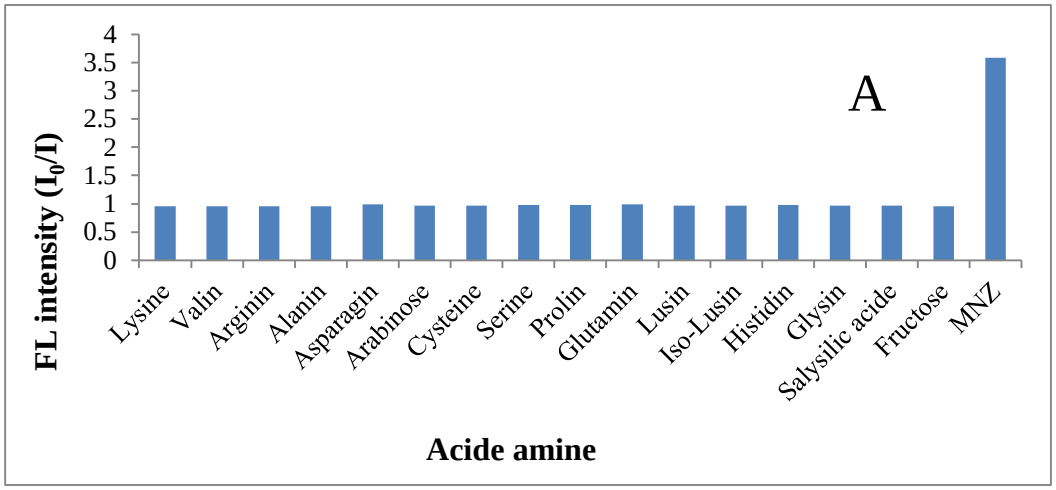
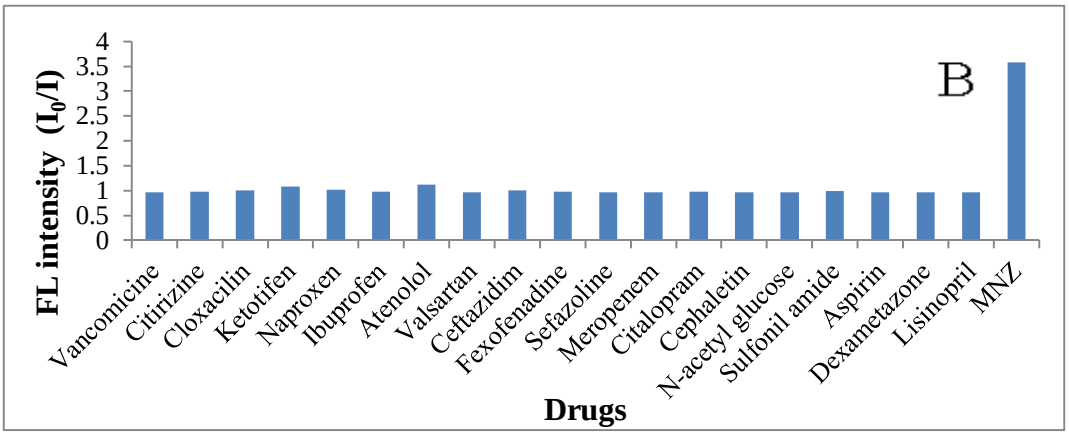


Fig.6. (A) Effects of pH on the fluorescence: the two curves represent the fluorescence intensity of QDs before (●) and after (■) the addition of MNZ, respectively.

3.4. Effect of reaction time: The reaction time was studied for the determination of metronidazole in the room temperature and the results showed that after 3 min of interaction between MNZ and C-dots maximal quench fluorescence intensity was observed after they were mixed together and the fluorescence signal was stable for at least 40 min. Thus, the fluorescence was measured after 3 min of adding of MNZ to pepper carbon dots.

3.5. Selectivity of the sensor: To evaluate the selectivity of the proposed sensors, the interference of ions including Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{3+} ,amino acids and some drugs such as (Meropenem, valsartan, Cetirizine, Ketotifen, Naproxen) and etc that might be present in real samples was investigated (**Fig.7**). It was found that 3.3×10^{-4} M concentrations of anions and drugs had little influence on the FL intensity of the sensor. The concentration of MNZ is 8×10^{-5} M.





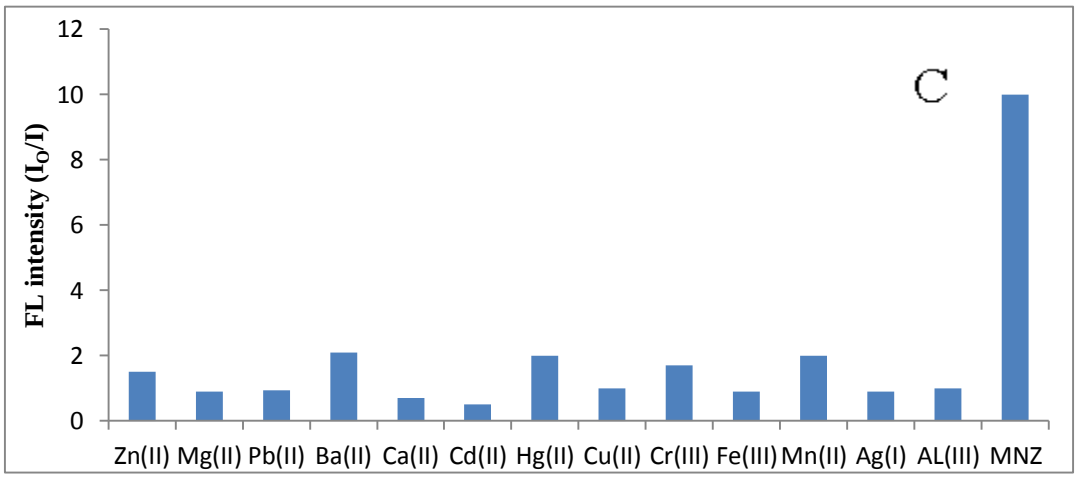
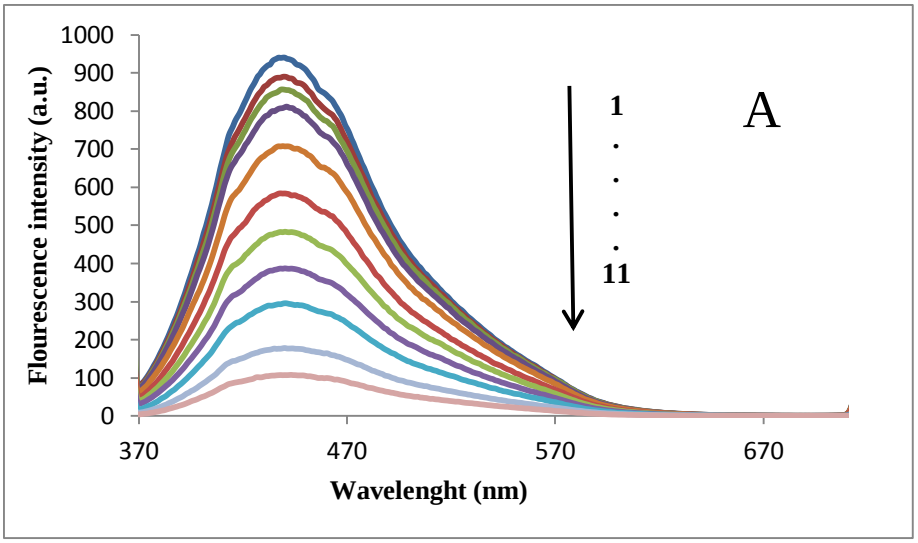


Fig.7. Effect of **A)** amino acids **B)** drugs and **C)** ions on the luminescence of pepper carbon dots. Concentrations of ions, amino acids and drugs are all 3.3×10^{-4} mol L^{-1}, and MNZ is 8×10^{-5}.

3.6. Fluorescence detection of metronidazole by the quenching emission of pepper CDs: The fluorescence intensity of the pepper C-dos decreased with the addition of MNZ, indicating a fluorescence quenching interaction between the C-dots and MNZ that is shown in (**Fig.8 A**). Under the optimum condition, the resulting plot (**Fig. 8 B**) shows a good linear dependence in the range from 5×10^{-9} molL^{-1} to 7.5×10^{-3} molL^{-1} with a correlation coefficient of 0.9918. For low metronidazole concentration. The limit of detection (LOD), determined using the equation $3\sigma/S$ (that σ is the standard deviation of blank measurements of 8 replicates and *S* is the slope of the calibration curve) is 2.9 nM. No emission peak shift was found even at relatively high concentrations of metronidazole.



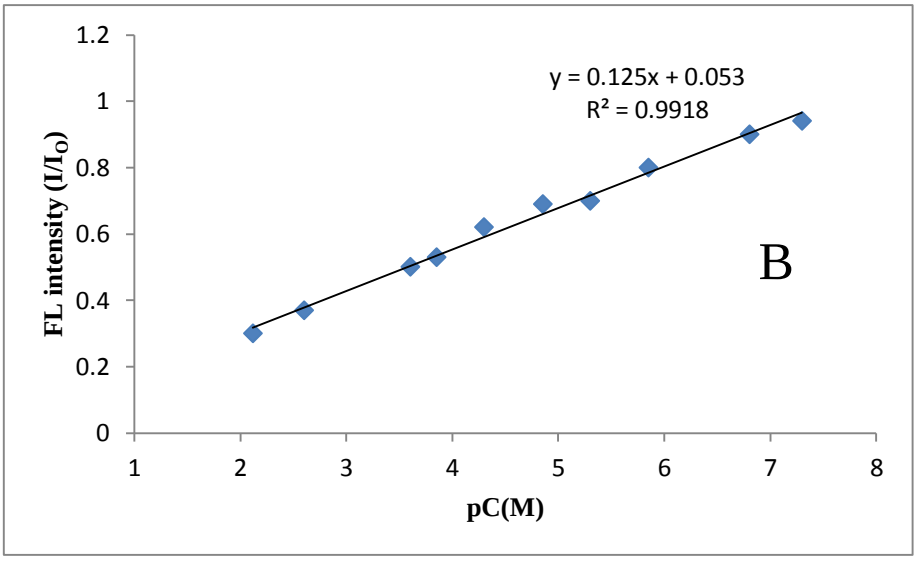


Fig.8. (A) Fluorescence spectra of C-dots in the presence of MNZ, from top to bottom, the concentrations of metronidazole **1)** 0 **2)**5×10^{-9} **3)**7×10^{-8} **4)**7×10^{-7} **5)**5×10^{-6} **6)**1.2×10^{-5} **7)**5×10^{-5} **8)**1.2×0^{-4} **9)**1.6×10^{-4} **10)**5×10^{-3}**11)**7.5×10^{-3}. **(B** Stern-Volmer plot the fluorescence intensity C-dots vs MNZ concentration.

3.7. Measuring metronidazole in real samples: To gauge the accuracy of our method for real sample analyses, the urine samples from 3 healthy volunteers were used as a clinical sample model. The results obtained by the proposed sensor were showed in **Table 2**. Good recoveries for the determination of MNZ were obtained in all cases, thus indicating the validity of the proposed method for direct analysis of MNZ in all samples.

Table 1. Determination of MNZ in real samples using pepper carbon dots.

Real samples	Recovery (%)	RSD (%)
Sample1	105	1.4
Sample2	101	2.1

CONCLUSION

In this work, pepper carbon dots were successfully synthesized and used as a sensitive and selective fluorescence probe for detection of metronidazole. In the presence of metronidazole, the fluorescence intensity of C-dots was decreased as the concentration of MNZ increased. The fabricated sensor showed good selectivity and sensitivity toward the detection of MNZ compared to other tested drugs, amino acids and metal ions. Additionally, this nanosensor has been successfully applied for the determination of metronidazole in real samples under the optimum conditions; the calibration plot was linear in the range between 5×10^{-9} molL^{-1} to 7.5×10^{-3} molL^{-1} and a correlation coefficient of 0.9918. The detection limit of this sensor is 2.9 nM. Therefore; this method is selective, sensitive, fast and simple for determination of metronidazole.

REFRENCES

1. Y.P. sun, B. Zhou, Y. Lin, W. Wang, K.A.S. Fernando, P. Pathak, M.J. Meziani, B.A. Harruff, X.Wang, H.Wang, P.G. Luo, H. Yang, M.E. Kose, B. Chen, L.M. Veca, S.Y. Xie, J. Am. Chem. Soc., 2006, 128, 7756-7757.
2. S.N. Baker, G.A. Baker, Angew. Chem. Int. Ed., 2010, 49, 6726–6744.
3. X. Y. Xu, R. Ray, Y .L. Gu, H.J. Ploehn, L. Gearheart, K. Raker, W. A. Scrivens, J. Am. Chem. Soc. 2004, 126, 12736–12737.
4. A.S. Athanasios, B. Bourlinos, Demetrios Anglos, RadekZboril, Vasilios Georgakilas, EmmanuelP. Giannelis, Chem. Mater.2008, 20, 4539–4541.
5. A.B. Bourlinos, A. Stassinopoulos, D.Anglos, R.Zboril, M.Karakassides, E.P.Giannelis, Small, 2008, 4, 455–458.
6. X.Y.Xu, R. Ray, Y.L. Gu, H.J. Ploehn, L. Gearheart, K. Raker,W. A. Scrivens, J. Am.Chem.Soc., 2004, 126, 12736–12737.
7. H. Liu, T. Ye, C. Mao, Angew. Chem. Int. Ed., 2007, 46, 6473–6475.
8. X.F. Jia, J. Li, E. K. Wang, Nanoscale, 2012, 4, 5572–5575.
9. Y. Feng, D.Zhong, H.Miao, X.Yang, Talanta. 2015, 140, 128–133.

10. X. Wang, L. Cao, S.T. Yang, F.S. Lu, M.J. Meziani, L.L. Tian, K. W. Sun, M.A. Bloodgood, Y.P. Sun, Angew. Chem. Int. Ed., 2010, 49, 5310–5314.
11. L. Cao, S. Sahu, P. Anilkumar, C.E. Bunker, J.A. Xu, K.A.S. Fernando, P. Wang, E.A. Guliants, K.N. Tackett, Y.P. Sun, J. Am. Chem. Soc., 2011, 133, 4754–4757.
12. H.X. Zhao, L.Q.Liu, Z.D. Liu, Y. Wang, X.J.Z hao, C.Z. Huang, Chem. Commun., 2011, 47, 2604–2606.
13. W.L. Wei, C. Xu, J.S. Ren, B.L. Xu, X.G. Qu, Chem. Commun., 2012, 48, 1284–1286.
14. L. Zhou, Y.H. Lin, Z.Z. Huang, J .S. Ren, X.G. Qu, Chem.Commun., 2012, 48, 1147–1149.
15. N. Samad, S. Masoum, B. Mehrara, H. Hosseini. Chromatography, 2015, 1001, 75–81.
16. C. Akay, S. Ozkan, Z. Senturk, S. Cevheroglu. Farmaco, 2002, 57, 953-957.
17. M·Farré, I. Ferrer, A.Ginebreda, M.Figueras, L.Olivella, L.Tirapu, M.Vilanova, D.Barceló. Chromatography, 2001, 938, 187–197
18. Z. Abbaspoor, Z. Rabee, S. Najjar.International J. Pharmacol. Res., 2014, 4, 78-83.
19. J. Muller, P. Schildknecht, N. Muller. J. Antimicrob. Chemoth., 2013, 68, 1781-1789.
20. J. Han, L. Zhang, S. Yang, J. Wang, D. Tan. B. Environ. Contam. Tox., 2014, 92, 96-201.
21. J.H. Wang. J. Chromatogr., 2001, 918, 435-438.
22. K.D. Trivedi, A.B. Chaudhary, S. Mohan. J. Adv. Pharm., 2013, 2, 05-11.
23. N.W. Ali, M. Gamal, M. Abdelkawy. J. Pharm. Sci., 2013, 26, 865-871.
24. J. Das, M. Dhua. J. Pharma. Sci. Tech., 2014, 3, 106-109.
25. V.R. Bari, U. Dhorda, M. Sundaresan, Anal.Chim. Acta, 1998, 376, 221-225.
26. P. Bartlett, E. Ghoneim, G. El-Hefnawy, I. El-Hallag. Talanta, 2005, 66, 869-874.
27. D. Chen, J. Deng, J. Liang, J. Xie, C. Hu, K. Huang. Sens. Actuators, 2013, 183, 594-600.
28. M.B. Gholivand, M. Torkashvand.Talanta, 2011, 84, 905-912.
29. W. Liu, J. Zhang, C. Li, L. Tang, Z. Zhang, M. Yang. Talanta, 2013, 10, 204-211.
30. Y.B. Mollamahale, M. Ghorbani, M. Ghalkhani, M. Vossoughi, A. Dolati. Acta, 2013, 106, 288-292.
31. Z. Wang, H. Zhou, S. Zhou. Talanta, 1993, 40, 1073-1075
32. Z. Yao, H. Jingbo, W. Zhongda, L. Qilong. Anal. Lett., 1998, 31, 429-437.
33. A. Salimi, M. Izadi, R. Hallaj, M. Rashidi. Electroanal, 2007, 19, 1668-1676.
34. J. Peng, C. Hou, X. Hu.Sens. Actuators, 2012, 169, 81-87.
35. E. Roy, S. K. Maity, S. Patra, R. Madhuri, P. K. Sharma.Adv., 2014, 4, 32881.
36. K. Nejati, K. Asadpour-Zeynali.Mat. Sci. Eng. C-Mater, 2014, 35, 179-184.
37. B. Yin, J. Deng, X. Peng, Q. Long, J. Zhao, Q. Lu, Q. Chen, H. Li, H. Tang, Y. Zhang and S. Yao, Analyst, 2013, 1-24.

Corresponding author: Naser Samadi;

Department of Chemistry, Faculty of Science, Urmia University, Urmia, Iran