Nano-Biosensor based on QDs for Ultrasensitive Detection of N-Methylamphetamine in urine samples

Hamideh Elmizadeh[1]
[1] *Department of Chemical Technologies, Iranian Research Organization for Science and Technology (IROST), 3313193685, Tehran, Iran*

ABSTRACT

In modern analytical chemistry, designing and fabricating various nano-biosensors based on quantum dots (QDs) is an interesting topic for the sensitive and rapid detection of different analytes. Sensitive detection of illicit drugs is important for maintaining global health. Among these drugs, rapid and easy detection of N-Methylamphetamine in urine is important due to its widespread abuse among young people.

In this work, the ligand exchange method was used to perform the surface modification of CdTe QDs with a specific aptamer. The size and morphology of the nano-biosensor were characterized by TEM and DLS. The prepared nano-biosensor had a spherical morphology and a size of about 5 nm. The prepared nano-biosensor is capable of measuring N-Methylamphetamine with a detection limit of 0.4 nM in real urine samples.

Keywords: *Nano-biosensor, QDs, illicit drug, urine samples*

1. *INTRODUCTION*

Quantum dots (QDs) have unique optical and electrical properties due to the quantum mechanical effects. QDs are used as fluorescence probes for the fabrication of various nano-biosensors[1-3]. Recently, the use of aptamers has been an ideal choice for the synthesis of various nano-bio sensors due to their advantages, including low cost, high selectivity [4, 5].

Sensitive and rapid analysis of the Illicit drug N-Methylamphetamine in urine samples is an important branch of modern forensic science[6,7]. Common analysis methods are used for this drug, such as HPLC [8], LC-MS/MS [9, 10], Raman spectroscopy [11], and so on. Although these methods have good accuracy, they have the same disadvantages as expensive equipment, pre-treatments of samples, and time-consuming analyses. In this work, CdTe QDs were synthesized with a TGA coating. Then, the nano-biosensor was fabricated using the ligand exchange method. The intensity of nano-biosensors is linearly quenched based on the PET mechanism by adding various concentrations of analyte. In addition, the prepared nano-biosensor determined the illicit drug in urine samples with satisfactory results.

2. *EXPERIMENTALT*

2.1 Materials and apparatus

Tellurium (Te) powder (99%), CdCl2·5H2O (99%), thioglycolic acid (TGA, 98%), and NaBH4 (95%) were obtained from Sigma (St. Louis, MO). All other chemicals used with analytical grade were prepared by Merck Chemical Co. DNA oligonucleotide (100 µmol L−1) purified by Hplc, was obtained from Pishgam Company (Tehran, Iran). Fluorescence studies were performed using a Fluorescence spectro fluorophotometer (Shimadzu RF-6000, Kyoto, Japan). TEM (transmission electron microscope, instrument Philips CM 10 H T, 300 kV, Japan) and DLS (dynamic light Scattering, Horiba- SZ100, Japan) were applied

to characterize the size and morphology of the nano- biosensor. FT-IR spectra were collected by Bruker Tensor 27 FT-IR spectrometer, Germany.

2.2 Preparation of CdTe-TGA QDs and nano-biosensor

CdTe-TGA QDs were synthesized based on our previously reported works [38-40]. For preparing the nano-biosensor, the optimized amount of QDs (20 µL, 2.5 µM) was added to RNase-free water (1 mL) under magnetic stirring (30 min) at room temperature. Then, the required amount of aptamer (20 µL, 0.6 µM) was dropwise added to the solution of QDs. The sealed system was kept for 24 hours under magnetic stirring (100 rpm). The prepared nano-biosensor can be stored without precipitation for 4 months at 4 ° C.

3. *RESULTS AND DISCUSSION*

3.1 Characterization

As shown in Fig. 1, the optimal time for nanobiosensor formation was 24 hours. The ligand exchange method leads to an enhancement of the QDs' fluorescence emission. This phenomenon may occur due to the formation of covalent bonds between donor atoms of sulfur and Cd2+ ions on the surface of CdTe QDs.

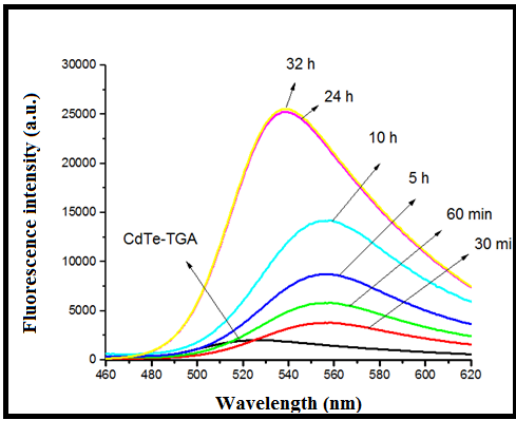

Fig. 1. The fluorescence spectra of CdTe-TGA QDs and the nano-biosensor during the ligand exchange method

IR spectroscopy in the near region was used to verify the formation of the nano-biosensor. As shown in Fig. 2, the peaks at 4345 and 5160 cm−1 are ascribed to the combination of S–H stretching and the combination of O–H stretching, respectively. Also, after the formation of the nano biosensor, peaks in the region 4000-5050 cm−1 were reduced due to the ligand exchange procedure.

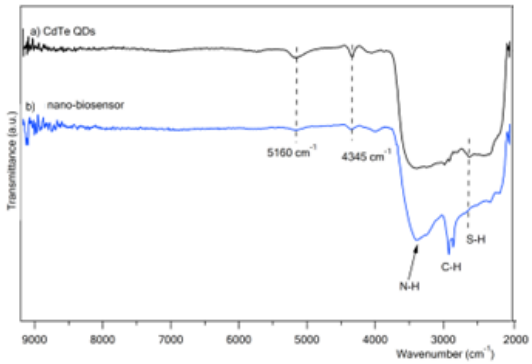

Fig. 2. FT-IR spectroscopy in the near region of QDs (a), and the nano-biosensor(b)

Transmission electron microscopy (TEM) was used to characterize the size and morphology of the prepared nano-biosensor. As shown in Fig.3a, the produced nano biosensor has approximately spherical nanoparticles with appropriate morphology and uniform distribution, with an average diameter of 7 ± 0.8. In addition, the dynamic light scattering (DLS) was used to determine the particle size of the nanobiosensor. As shown in Fig. 3 b, the particle size of the nano-biosensor was 8 ±1.7.

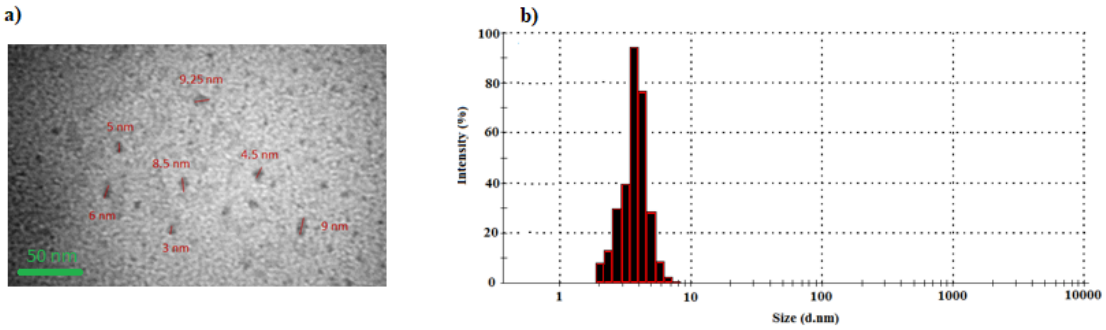

Fig. 3 TEM image (a) and DLS of the prepared nano-biosensor

3.2. Measurement of the illicit drug and calibration curve

As shown in Fig.4, after adding various amounts of the target, the fluorescence intensity of the prepared nano biosensor was remarkably decreased. This quenching of nano-biosensors may be performed based on a type of photo-induced electron transfer (PET) mechanism. PET is a reversible quenching process based on the excited electron transfer after generating an electron-hole pair, from the conduction band to an electron acceptor or accepting an electron in the hole from a donor [12]. After the formation of the aptamer-N-Methylamphetamine complex, based on the PET mechanism, the excited electron is transferred from CdTe QD (donor) to N-Methylamphetamine (receptor). The Stern–Volmer Eq. (1) was used for the analysis of its quenching.

$$\frac{F_0}{F} = 1 + K_{sv}[Q] \qquad (1)$$

In this equation, F0 and F are the fluorescence emission signals of the nano biosensor at 550 nm in the lack and existence of a quencher (N-Methylamphetamine), respectively. Ksv is the quenching constant of the quencher, and [Q] is the analyte concentration. The Stern–Volmer equation is Y= 12.33 X + 1 with a

determination coefficient (R^2) of 0.99. The linear range is 0.13 ± 0.25 × 10^{-9}– 0.12 ± 0.07 × 10^{-6} mol L^{-1}, and the detection limit, (LOD=3Sb/slope, S/N = 3) is 0.41 ± 1.05 × 10^{-9} mol L^{-1} (Fig. 5).

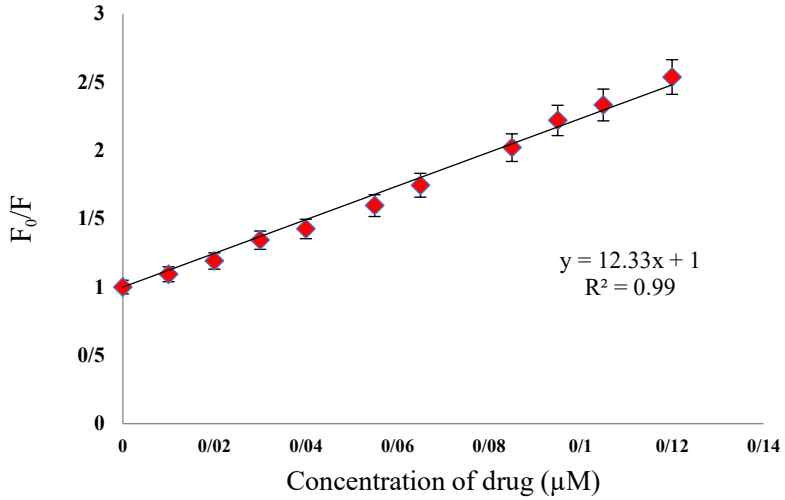

Fig. 4 Stern-Volmer plot

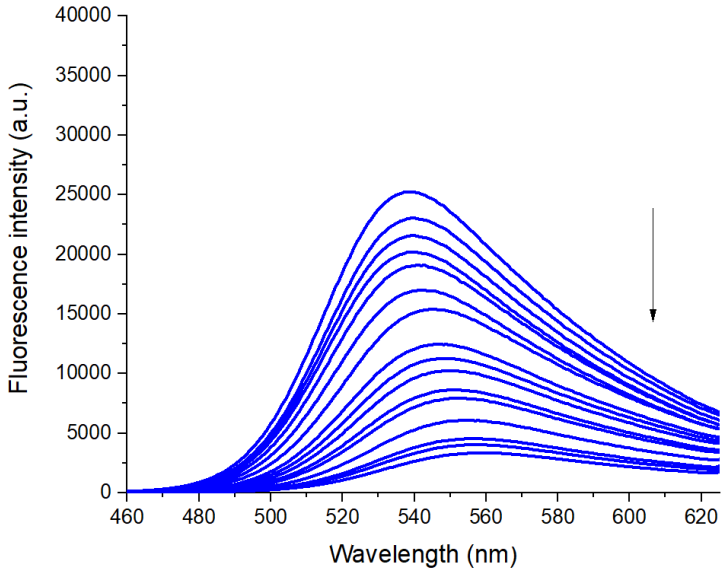

Fig. 5 Fluorescence titration of prepared nano-biosensor solution after adding various concentrations of analyte

3.3. Specificity and selectivity study

One of the essential parameters in evaluating sensor performance is its selectivity for the specific analyte. Therefore, the optical response of the nano-biosensor to some of the common interferences in biological surroundings such as Arginine, Tryptophan, Valine, Lysine, Alanine, Asparagine, Glycine, Ca^{2+}, K^{+}, Na^{+} and other drugs such as morphine, amphetamine, and cocaine (10 times higher concentration than the analyte) were investigated. As shown in Fig.6, the synthesized nano-biosensor has good selectivity in detecting the analyte.

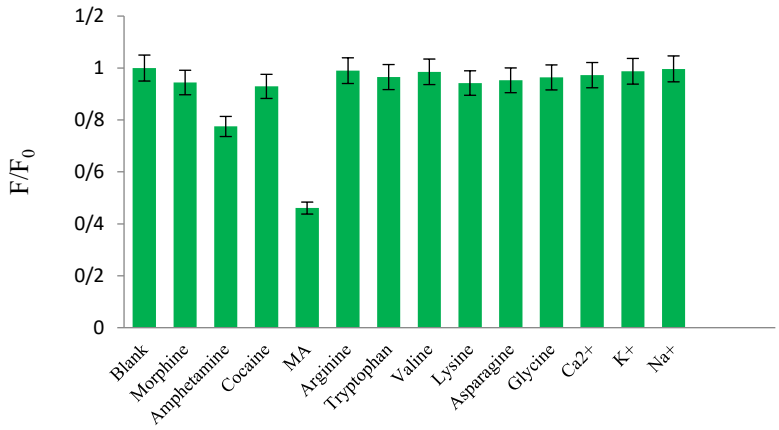

Fig. 6 Selectivity of apta-nano biosensor toward *N-Methylamphetamine*

3.4. Determination of N-Methylamphetamine in the real urine samples

The ability of the nano-biosensor to analyze real urine samples was investigated. Initially, the urine samples were prepared by filtration (Syringe filter (0.2 µm)) and centrifugation (10,000 rpm for 5 min). Then, the obtained clear solution was diluted with distilled water (10 times) and was analyzed using the spike method. As shown in Table 1, the recovery of N-Methylamphetamine was between 97.19 % and 102.6 4% with RSDs lower than 2.75 %. The obtained results proved that the introduced nano-biosensor is capable of rapid and easy detection of the analyte in a complex matrix.

Table 1. Determination of *N-Methylamphetamine* in urine samples according to the spike method (n=5)

Sample	Added (µM)	Found (µM)	Recovery (%)	RSD (%)
Urine	0.005	0.0048	97.2	2.1
Urine	0.015	0.0145	97.1	2.5
Urine	0.025	0.0249	99.8	1.4
Urine	0.045	0.0461	102.6	2.7

4. Conclusion

Herein, the nano-biosensor based on surface modification of specific aptamer-coated quantum dots was designed and fabricated. Then, the selectivity and efficiency of the nano-biosensor were investigated in real urine samples, and satisfactory results were obtained. The nano-sensor fluorescence was quenched by adding various concentrations of the illicit drug based on a type of PET mechanism. Under optimal conditions, a linear range was obtained in the calibration plot. The detection limit was 0.4 nM, and a correlation coefficient (R2) of 0.99 was achieved.

REFERENCES

[1] *H. Elmizadeh, M. Soleimani, F. Faridbod, G. Bardajee, Fabrication of a nanomaterial-based fluorescence sensor constructed from ligand capped CdTe quantum dots for ultrasensitive and rapid detection of silver ions in aqueous samples, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 211 (2019) 291-298.*

[2] *H. Elmizadeh, M. Soleimani, F. Faridbod, G.R. Bardajee, Fabrication and optimization of a sensitive tetracycline fluorescent nano-sensor based on oxidized starch polysaccharide biopolymer-capped CdTe/ZnS quantum dots: Box–Behnken design, Journal of Photochemistry and Photobiology A: Chemistry 367 (2018) 188-199.*

[3] *H. Elmizadeh, M. Soleimani, F. Faridbod, G.R. Bardajee, A sensitive nano-sensor based on synthetic ligand-coated CdTe quantum dots for rapid detection of Cr (III) ions in water and wastewater samples, Colloid and Polymer Science 296(9) (2018) 1581-1590.*

[4] *M. Heiat, A. Najafi, R. Ranjbar, A.M. Latifi, M.J. Rasaee, Computational approach to analyze isolated ssDNA aptamers against angiotensin II, Journal of Biotechnology 230 (2016) 34-39..*

[5] *L. Chen, N. Xia, T. Li, Y. Bai, X. Chen, Aptasensor for visual and fluorometric determination of lysozyme based on the inner filter effect of gold nanoparticles on CdTe quantum dots, Microchimica Acta 183(11) (2016) 2917-2923.*

[6] *S. Mizuno, S. Ono, H. Yasunaga, H. Iwase, Factors associated with methamphetamine use in Japanese people who died of unnatural causes: analysis using forensic autopsy reports, Forensic Science International 327 (2021) 110946.*

[7] *S.J. Brookfield, L. Selvey, L. Maher, L. Fitzgerald, "It Just Kind of Cascades": A critical ethnography of methamphetamine-related pleasure among people in recovery, International Journal of Drug Policy 98 (2021) 103427.*

[8] *M. Ebrahimi Rahmani, M. Ansari, M. Kazemipour, M. Nateghi, Selective extraction of morphine from biological fluids by magnetic molecularly imprinted polymers and determination using UHPLC with diode array detection, Journal of separation science 41(4) (2018) 958-965.*

[9] *J.M.M. Kahl, K.F. da Cunha, L.C. Rodrigues, K. de Oliveira Chinaglia, K.D. Oliveira, J.L. Costa, Quantification of amphetamine and derivatives in oral fluid by dispersive liquid-liquid microextraction and liquid chromatography—tandem mass spectrometry, Journal of Pharmaceutical and Biomedical Analysis 196 (2021) 113928.*

[10] *M.K. Green, A.L. Ciesielski, J.R. Wagner, Detection of one pot methamphetamine laboratory byproducts in wastewater via solid phase extraction and liquid chromatography-tandem mass spectrometry, Forensic Chemistry 19 (2020) 100253.*

[11] *N. Nuntawong, P. Eiamchai, W. Somrang, S. Denchitcharoen, S. Limwichean, M. Horprathum, V. Patthanasettakul, S. Chaiya, A. Leelapojanaporn, S. Saiseng, Detection of methamphetamine/amphetamine in human urine based on surface-enhanced Raman spectroscopy and acidulation treatments, Sensors and Actuators B: Chemical 239 (2017) 139-146.*

[12] *J.F. Callan, R.C. Mulrooney, S. Kamila, B. McCaughan, Anion sensing with luminescent quantum dots–a modular approach based on the photoinduced electron transfer (PET) mechanism, Journal of fluorescence 18(2) (2008) 527-532.*