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ORIGINAL ARTICLE

A Novel Fluorescence Nanobiosensor based on Modified Graphene Quantum dots-HTAB for Early Detection of Fetal Sexuality with Cell Free Fetal DNA

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Abstract
Recently, prenatal diagnosis with non-invasive insight is a progressive approach in clinical medicine to prevent the birth of infants with genetic abnormalities. Cell free fetal DNA (cffDNA) makes up approximately 3–6% of the bare DNA in the mother's bloodstream which is produced during pregnancy and can be used to detect fetal sex and disease in the early stages. SRY is a gene located on the chromosome Y which determines the sex of male infants. In this work, a new nanobiosensor based on the fluorescence property of r-GQD@HTAB (reduced graphene quantum dots modified with hexadecyl trimethyl ammonium bromide) was fabricated that can identify the SRY gene in cffDNA with high sensitivity and specificity. A detection limit of 0.082 nM and the linear response range of 0.16–1.5 nM was obtained for the method. It was able to discriminate the target sequence with high specificity from the non-target sequences. This biosensor includes a new graphene quantum dot modified with a surfactant, HTAB which leads to high fluorescence emission of it and then more precise differentiation between ssDNA and DsDNA in a solution. In conclusion, it provides a novel analytical tool for detection of small amount of DNA and fetal sex and genetic diseases in early stage with prenatal and noninvasive tests and applicable for clinical use.

Keywords Nanobiosensor · Fluorescence · Sex determination · SRY gene · cffDNA · r-GQD@HTAB

Introduction

To determine the genetic sex of fetus in human, animal models and livestock, especially in the early stages of their formation in the mother's body, is an important issue. This is due to certain effects on growth and development related to gender, as well as some genetic effects and certain diseases are correlated to the fetus sex. In humans and mice, the male or female sex is influenced by the SRY gene, which is located on the chromosome Y [1, 2].

In general clinical methods for sex determination, it used amniocentesis or chorionic villus sampling as invasive techniques and ultrasonography as well that cannot be performed until the first trimester due to uncompleted development of the external genitalia [3]. Therefore, the use of new techniques for fetus sex determination especially with using the analysis of fetal genetic material obtained from the fetal cells DNA in mother blood as a noninvasive method can help clinicians in the early stage of pregnancy. The discovery of cell free fetal DNA (cffDNA) fragments in the blood plasma of mothers in 1997 was opened the way to genetic analysis of fetus sex and diseases with testing mother's blood sample [4].

A routine technique for gene and nucleic acid detection in blood is PCR. However, the technique includes a substantial amplification error during the duplication process due to Taq polymerase activity. An alternative solution for the techniques based on PCR is the application of biosensors that can improve the problem with high accuracy, a decrease of recognition cost, and high speed. A biosensor as an analytical tool qualifies and quantifies biological and biochemical reactions through the exchange of them to electrical, optical or other kinds of signals [5].

Fluorescent nanoparticles such as quantum dots [6], dye-doped silica nanoparticles [7], carbon dots [8] and so on have

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been frequently used as fluorescent tags and also to provide high sensitivity associated with colorimetric detections and biosensing [9]. Recently, graphene quantum dots (GQDs) due to many characteristics includes a size of nanometer-scale and a single or few-layer graphene's structure, low cost, biocompatibility, low cytotoxicity, rich functional groups, etc. have received enormous attraction [10–12].

In this study, it was developed a new nanobiosensor based on the fluorescent reduced graphene quantum dots modified with hexadecyl trimethyl ammonium bromide (r-GQD@HTAB) nanoparticle to identify the SRY gene sequence in cffDNA with high sensitivity and specificity. Our sensor has advantages over published graphene quantum dots based biosensors, where a surfactant (HTAB) is linked to the surface of r-GQD and this can potentially lead to high fluorescence emission of the nanoparticle and exact discrimination between single and double stranded DNA for interaction with r-GQD. Hence, it can be an effective analytical tool for detection of small amount of DNA in prenatal and noninvasive tests.

Materials and Methods

Chemicals and DNA Oligonucleotides

All chemicals including citric acid, sodium hydroxide, hydrochloric acid, Tetrahydrofuran, and sodium borohydride purchased from Merck (Germany) in analytical reagent grade and it used without any further purification. All used solutions were prepared in deionized water (ddH_2O), except for the reaction solution for biosensor performance. The SRY gene sequence (Gene Bank accession no. NG_000007) was used to design synthetic oligonucleotides. The oligonucleotides were synthesized by Macrogen Inc. (Seoul, Republic of Korea) in HPLC purified lyophilized powder, and then for use, it dissolved in ultrapure water or buffer. The oligonucleotide used in this work were as follow:

The probe (**P**: 5- ACTTAGAGTTACAGCTTTCAGTGC -3), the target sequence (**T**: 5-GCACTGAAAGCTGTAACT CTAAGT-3), and non-complementary base with mismatch sequences (**MIS I**: 5- GCGCTGAAAGCTGTAACTCTA AGT-3 and **MIS II**: 5- GCGCTGAAAGCTGTAACTCTA ATT-3).

Instruments

Ultraviolet–visible absorption spectra measured using A Lambada 25 PerkinElmer Spectrometer (Hewlett Packard Corporation, Palo Alto, CA, USA). Fluorescence spectra was recorded using an LS50 PerkinElmer Spectrofluorometer and Felix Software (Photon Technology International, Lawrenceville, NJ, USA). Transmission Electron Microscope (TEM) (ZEISS EM 900, Germany) and Field Emission Scanning Electron Microscope (FESEM) (TESCAN mira2, Czech Rep.) microscopes used for imaging and size measurement. A PerkinElmer (USA) instrument did FT-IR spectroscopy.

Preparation of r-GQDs and Modification with HTAB

The direct pyrolysis method was used to synthesize GQDs from Citric acid. Briefly, 2 g of Citric acid powder heated to 200 °C. After 30 min, the obtained orange liquid was added drop by drop into 100 mL of NaOH solution (10 mg/mL) under vigorous stirring. After decreasing to pH 7.0 (with NaOH), the aqueous solution of GQDs was synthesized. To prepare r-GQD, it was dissolved 1.63 g of GQD and 0.1 g of sodium borohydride in 20 ml of tetrahydrofuran solution. The solution was placed at 70 °C for 8 h and then, was left in oven at 100 °C overnight for drying. The resultant was completely dried and finally, the r-GQD obtained as powder. To modify r-GQD with HTAB, first, it was dissolved the r-GQD in 50 ml of ddH_2O. The pH of the solution was reduced to 7 to protonate the carboxyl groups. Per 3 ml of the solution, it was added 7 mg of HTAB and kept for 1 h with shaking for the formation of r-GQD@HTAB.

Characterization of Nanoparticles

To determine the size of r-GQD@HTAB and to characterize it, a high magnification TEM and FESEM microscope were used. First, it was solved a little bit of nanoparticles with distilled water in a vial, sonicated for 20 min to spread the nanoparticles, then 100 µl of it was placed on a slide and dried under an IR lamp. In follow, after coating with gold, it was collected images under the microscope. A PerkinElmer spectra instrument was applied for infrared analysis and getting FTIR spectra of nanoparticles.

Sensitivity and Selectivity of the Nanobiosensor

Briefly, 50 µl of probe (1 µM) was heated in a water bath at 95 °C for 2 min (to open the DNA coil), and then, it was placed at room temperature for 1 h, in follow, the r-GQD@HTAB was added. Subsequently, different concentrations of the target sequence were added to determine the sensitivity of biosensor. Hybridization of probe and target sequences was performed at 37 °C for 15 min. After that, the samples were analyzed by a spectrofluorometer to evaluate fluorescence emission intensity and to obtain the limit of detection in the presence of different concentrations of the target sequence. Titration was performed to assess the linearity response range of the nanobiosensor in different volumes and concentrations. The limit of detection (LOD) was calculated based on the formula, $LOD = (F \times SD)/b$, which SD

is the standard deviation, b is the slope of the line and F is equal to 3.3.

To assay the selectivity of the method, 150 µl of each noncomplementary sequence (1 nM) containing one or more mis-match bases used for hybridization with the probe, separately.

To evaluate the capability of the innovative method for use in detecting sequences in real clinical samples, a healthy female blood plasma sample with an O^+ blood group was prepared from the Blood Transfusion Organization. Real sample analysis was performed by the spike method. To remove heavy proteins, 2 ml of 1 M nitric acid/1 ml of plasma was added and the separation of supernatant was done by centrifugation at 4000 rpm. The supernatant was used as a real sample to measure the performance of biosensor.

Results and Discussion

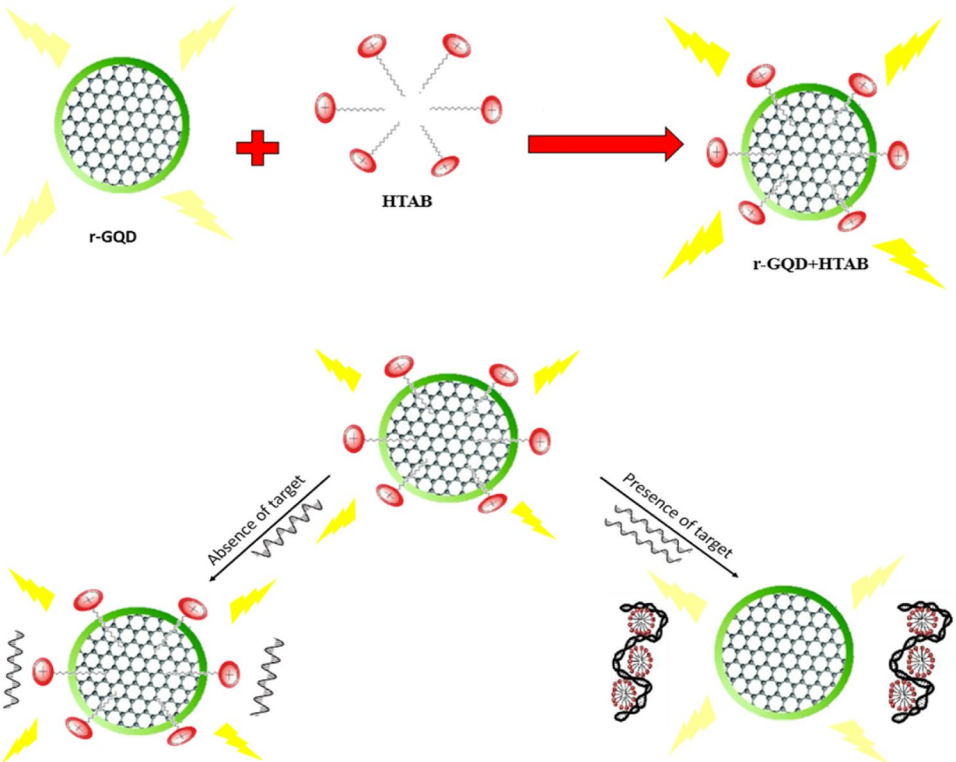
The Principle of r-GQD@HTAB-based Nanobiosensor

Reduced graphene quantum dots have high fluorescence emission and optical stability compared with GQDs and then it can be used as a fluorescent reporter in sensors and establishment of detection methods by controlling its emission. The basis of our work is to reduce the fluorescence emission of r-GQD@HTAB nanoparticles in the presence of the target sequence. Indeed, in this work using the fluorescence property of r-GQD nanoparticles modified with cationic surfactant (HTAB) and the ability of HTAB for bind to double-stranded DNA and subsequently, reduce the nanoparticle emission, a model of sensor is proposed that it can detect single stranded DNA from double stranded DNA. This characterization can be used to identify SRY gene sequence in cffDNA with low concentration and high specification. The modification of nanoparticles with HTAB increases its fluorescence emission intensity. Scheme 1 shows the basis of our label-free fluorescence strategy.

The High Magnification Microscopy imaging and FT-IR spectra

The morphology and size of the r-GQD nanoparticles were carefully characterized with TEM and FESEM. Figure 1 illustrates the results which the nanoparticle size of r-GQDs is less than 10 nm in diameter with a circular shape and a crystal lattice structure. The result confirmed that the nanoparticles had been successfully synthesized.

FTIR was performed as a confirmatory analysis for reduction of GQD to r-GQD. Figure 2 shows the FTIR spectra of r-GQD and GQDs. As shown in this figure, it can be observed the characteristic peaks related to functional groups. A peak at ~1700 nm in the spectra corresponds to CO (carbonyl) bond formation.



Scheme 1 The principle of target detection by r-GQD@HTAB-based nanobiosensor

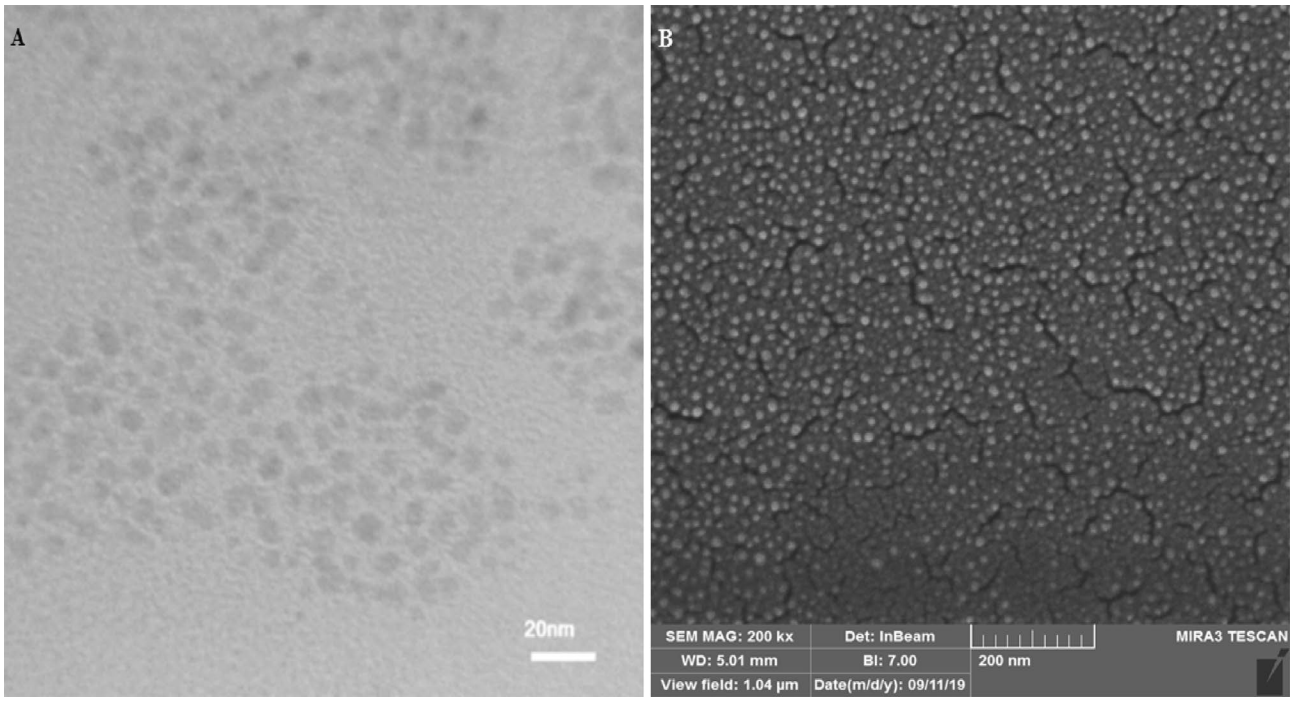


Fig. 1 The Transmission Electron Microscope (**a**) and the Field Emission Scanning Electron Microscope (**b**) image of r-GQDs

Fluorescence and UV Absorbance Spectroscopy

The fluorescence emission of GQDs and r-GQDs was investigated with excitation at 378 nm. Figure 3 shows the emission intensity at 450 and ~ 460 nm spectra for GQD and r-GQD, respectively. As shown in the figure, the fluorescence emission spectrum of r-GQD has shifted to longer wavelength.

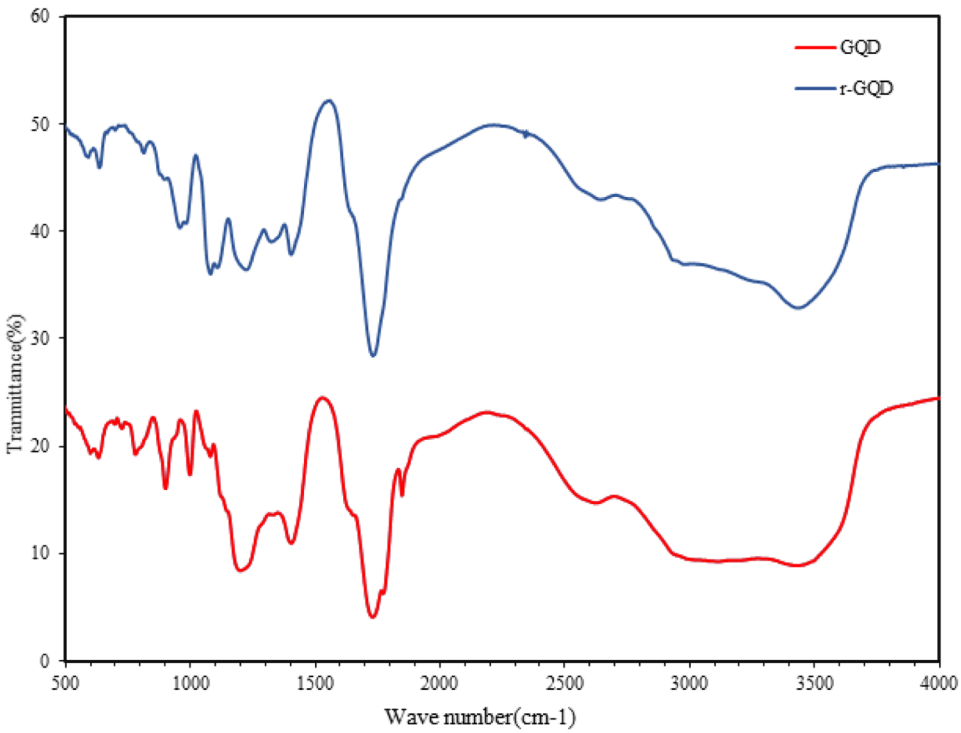


Fig. 2 The FTIR spectra of GQDs and r-GQDs

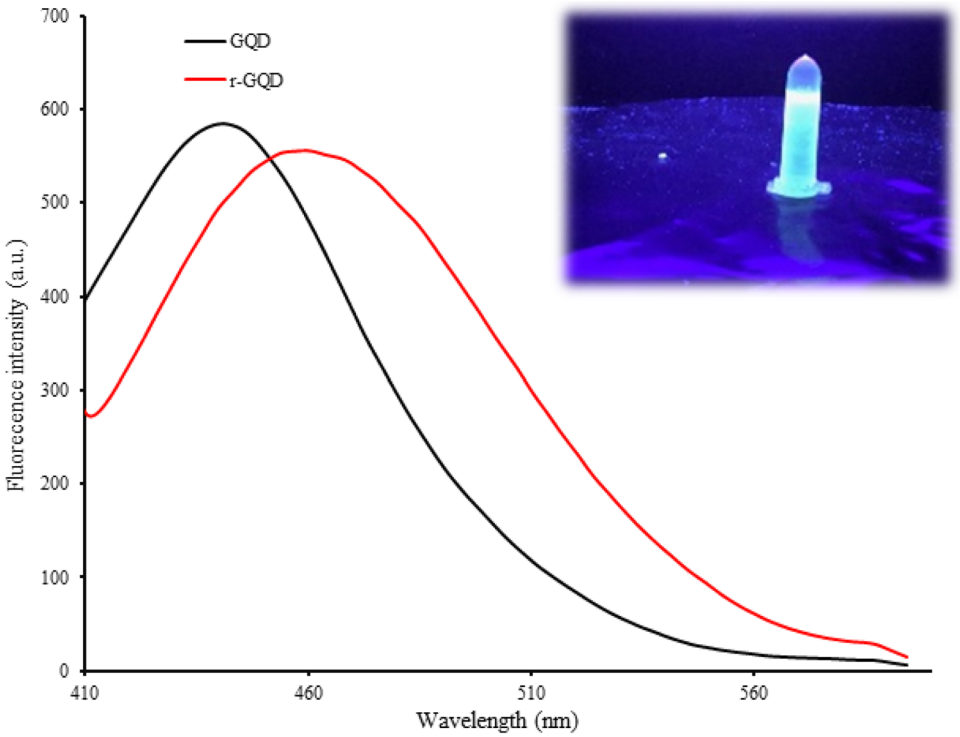


Fig. 3 The fluorescence spectra of GQDs and r-GQDs. r-GQDs in a dark room under a 360 nm UV lamp (inlet)

It was recorded the UV–vis peaks from wavelength 280 to 600 nm. Figure 4 indicates the ultraviolet–visible spectra of GQDs and r-GQDs. As shown in this figure, GQDs and r-GQD both displayed absorption peaks both at 280 nm. Besides, at the r-GQD absorption edge, there is little displacement along the wavelength.

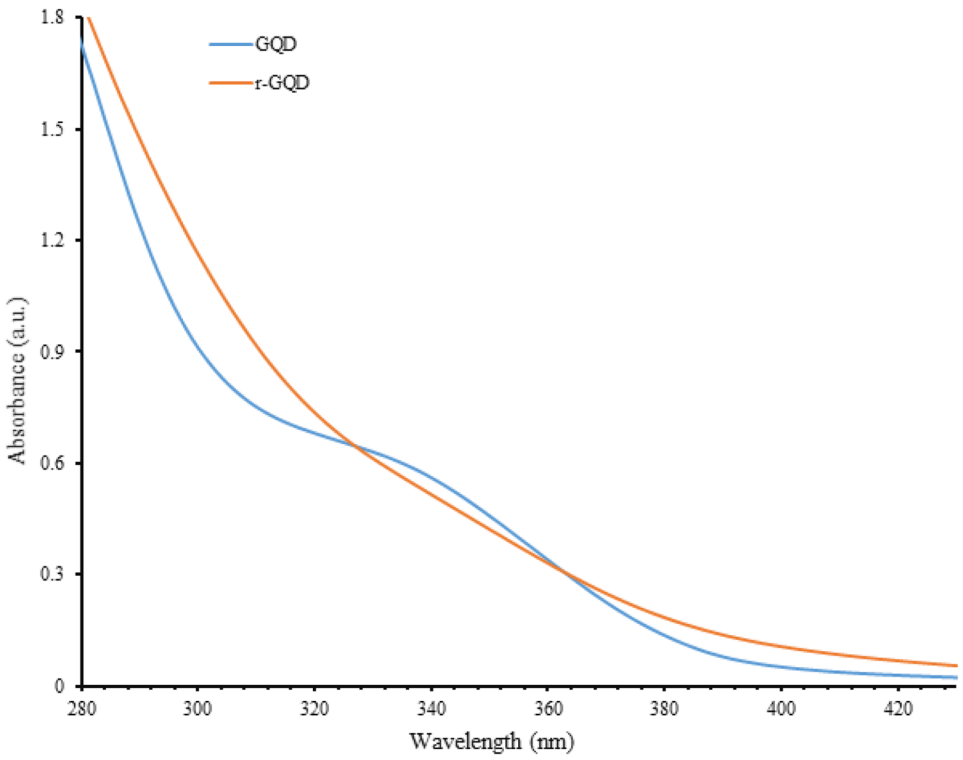


Fig. 4 The UV absorbance of GQD and r-GQDs

Different Fluorescence Emission of GQD and r-GQD with and without HTAB and DNA

To confirm our methodology and biosensor principle, different concentrations of GQD were prepared and its fluorescence emission intensity was measured, and then incubated under conditions including different temperatures and times with different concentrations of probe and target sequences. No emission change was observed. The probe could not attach to the GQDs as an identifying agent and it was not observed any change in fluorescence intensity in the presence of the target sequence. Figure 5a shows a lack of significant change in the fluorescence spectrum due to no interaction between synthesized GQDs with probe and the different concentrations of target sequence.

The GQDs are non-toxic and have high quantum gain and solubility in water, but because they are negatively charged due to carboxylic groups, they did not interact with the DNA probe during the experiments. Therefore, it was used HTAB cationic surfactant to investigate the change in GQDs emission in the presence of the surfactant and nucleotide sequences. In this case, also the changes in emission were not noticeable and were not reproducible (Fig. 5b).

In the other experiment, it was reduced GQD to r-GQD to get a sufficient result with nucleic acids sequences (probe and target). Figure 5c and d show the fluorescence spectra shift and significant intensity of r-GQD in presence of HTAB (r-GQD@HTAB) and probe-target sequences, respectively.

Optimization of Experimental Parameters

To obtain a better biosensing performance and getting clear validity, it was measured and optimized different parameters, including effect of pH, HTAB, probe concentration and time duration on r-GQD fluorescence intensity. Figure 6 presents the results of these optimizations.

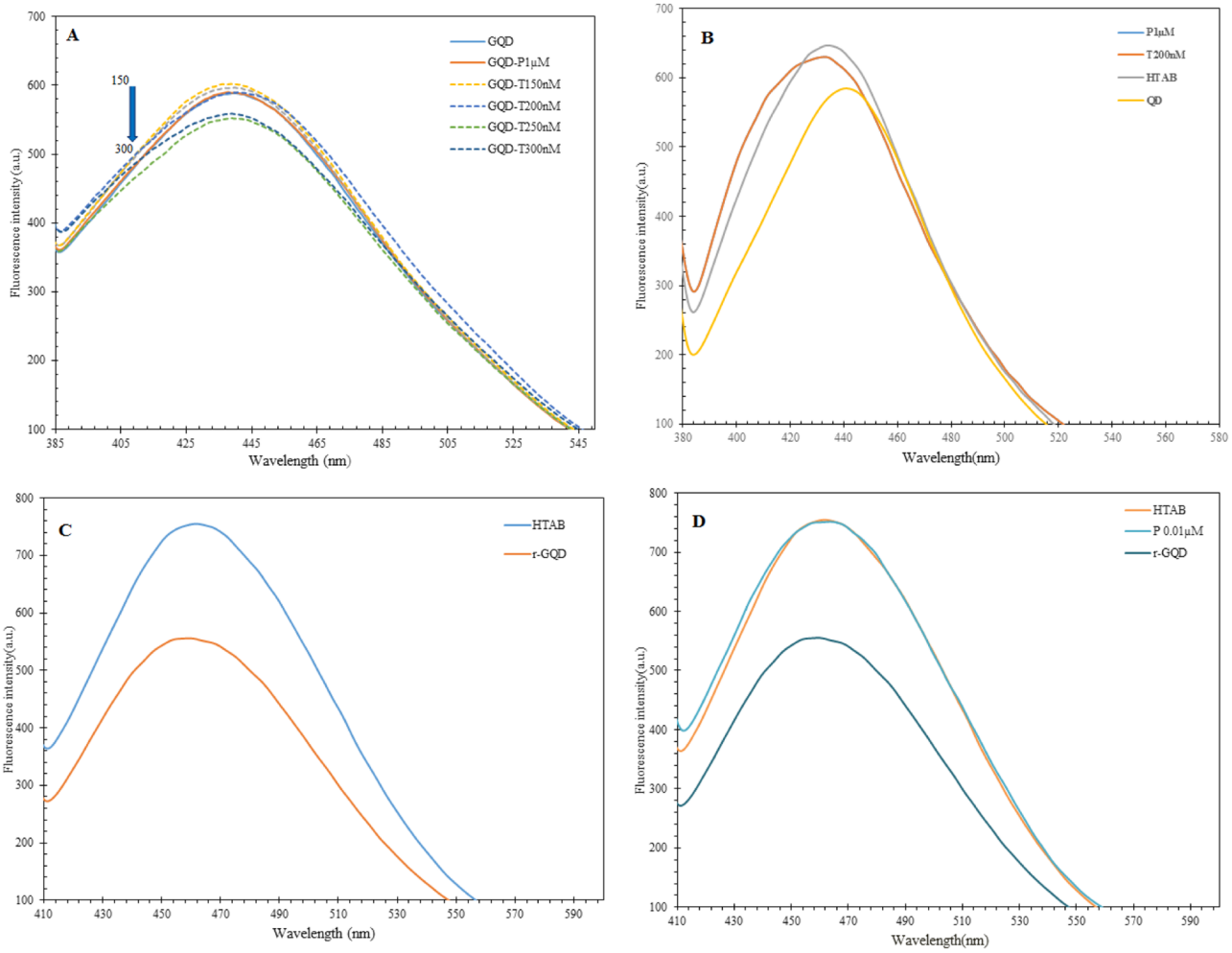


Fig. 5 The fluorescence intensity by the interaction of GQDs with probe-target sequences (**a**), HTAB-probe-target sequences (**b**), and r-GQDs with HTAB (**c**) and HTAB-probe-target sequences (**d**)

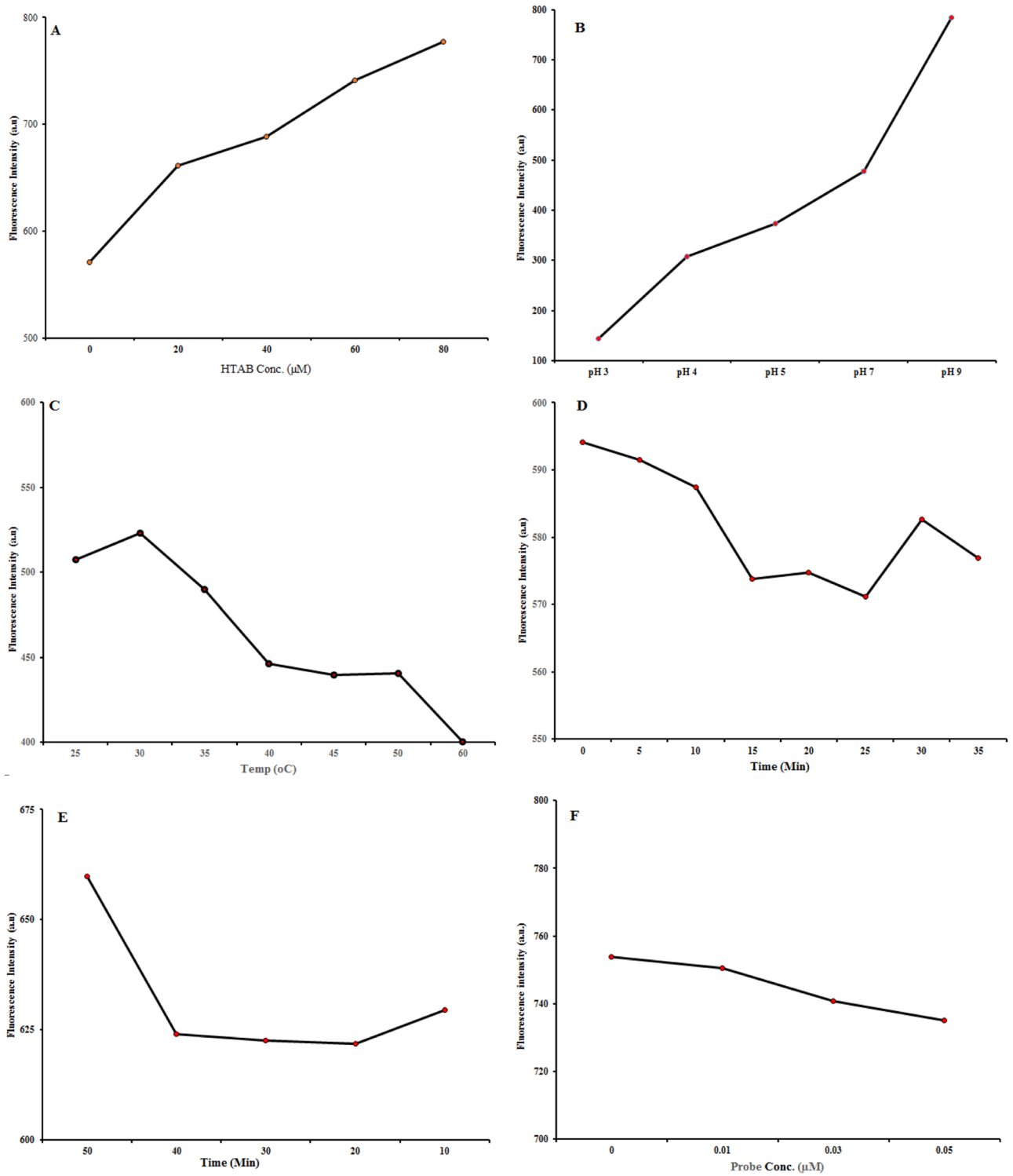


Fig. 6 The effect of different parameters on fluorescence intensity of r-GQD. The HTAB concentrations from 20 to 80 μM (**a**), pH (3–9) (**b**), Different temperatures after adding Target (25–60 °C) (**c**) Time after adding probe to r-GQD@HTAB (0-35 min) (**d**), Time after adding HTAB to r-GQD (10-50 min) (**e**), and different concentration of probe before adding target sequence (0–0.05 μM) (**f**)

The different concentrations of HTAB (20–80 μM) were added to a constant amount of r-GQD nanoparticles. As shown in Fig. 6a, with increasing concentration of HTAB, fluorescence intensity increases, significantly. The effect of different pHs of PBS buffer on the fluorescence intensity of r-GQD was investigated (Fig. 6b). The fluorescence

emission of r-GQD is strongly influenced by pH so that the intensity of fluorescence emission increases from acidic to alkaline pH. This behavior can be explained by the structure of r-GQD. At acidic pHs, the empty sites of the zigzag edges become protonated. Therefore, the emitting carbon is lost and inactivated in terms of fluorescence [13]. The best emission of r-GQD was at pH = 7, which is the neutral pH. As shown in the Fig. 6c, with increasing temperature from 30 °C, the emission intensity decreased. It seems the best time for a complete hybridization probe-target and obtaining the highest emission for r-GQD@HTAB is about room temperature (30 °C).

To obtain the appropriate time to study, the effect of time on efficiency and the fluorescence emission intensity of r-GQD was investigated at different times after the addition of the probe sequence (Fig. 6d) and HTAB (Fig. 6e). The best time with balanced emission was 10 to 35 and 40 to 50 min respectively after adding surfactant (HTAB) and probe to the reaction solution (containing r-GQD@HTAB).

Besides, the optimized time for hybridization of probe-target sequences (in solution includes of r-GQD@HTAB) was 20–25 min. The effect of probe concentration was investigated by using different concentrations (0.01, 0.03, and 0.05 μM) of the probe and a constant concentration of r-GQD@HTAB. No significant change was seen with increasing probe concentration (Fig. 6f).

The Analytical Performance of Nanobiosensor

The Sensitivity Assay

To evaluate the biosensor sensitivity, various concentrations of target sequence were prepared in the presence of 50 μl of probe (1 μM) and r-GQD@HTAB into a fluorescence cell. As it can be seen in Fig. 7a, with the increase in concentration of target sequence, the fluorescence emission intensity is decreased which confirms a signal off mode for the biosensor.

It was obtained a steady response for the method up to the concentration of 1.5 nM. A linearity range of 0.16–1.5 nM ($R^2 \sim 0.99$) with a LOD of 0.082 nM was obtained for the biosensor (Fig. 7b) which these values were in high accordance with the linear range of calibration cure obtained in the Fig. 7a.

Based on the scientific literature, the concentration range of fetal cffDNA in maternal blood is 4% to 15% of cffDNA with mostly about 14.4% [14, 15] (average of cffDNA in the mother's blood is ~ 13–19 ng/ml [16]). Thus, it found the fluorescence-based nanobiosensor has a persuadable

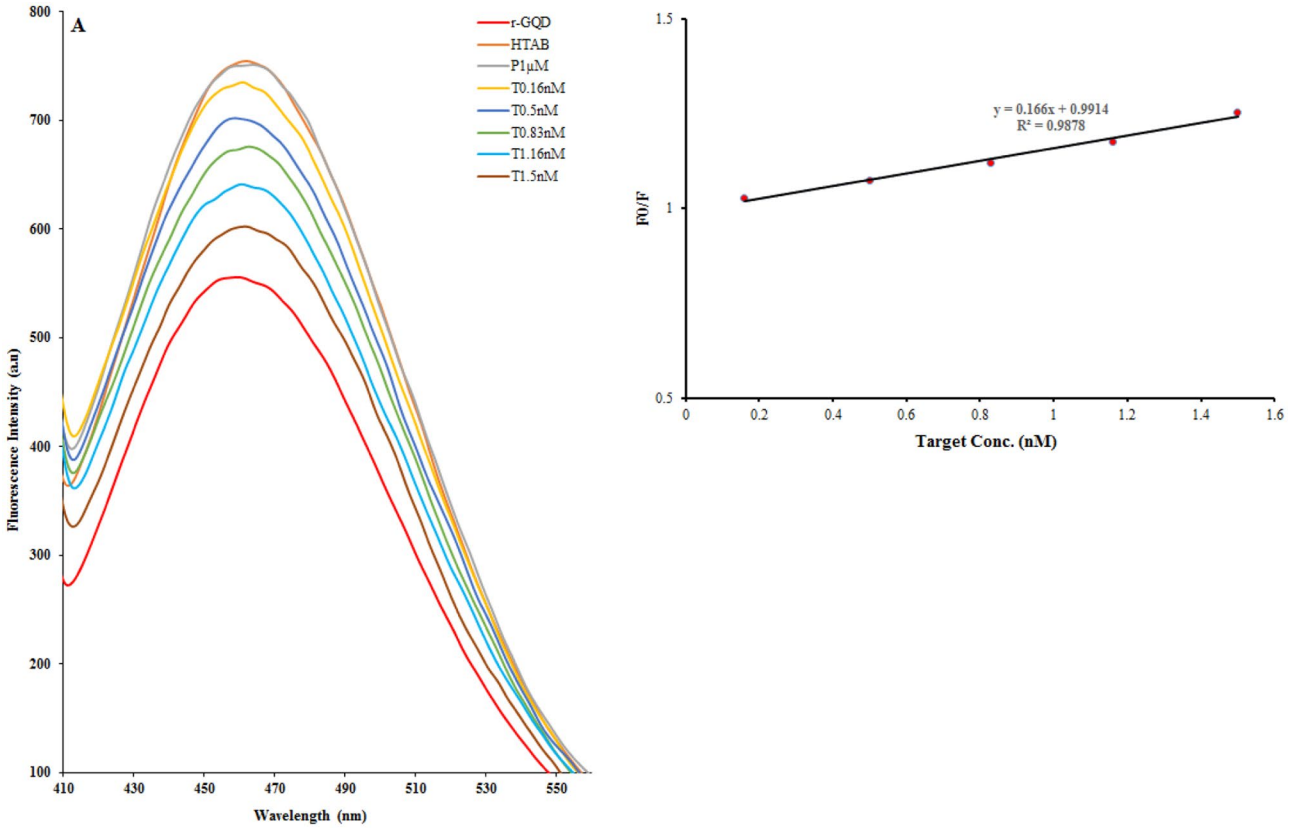


Fig. 7 The decreasing of fluorescence intensity with the increase of target concentration (**a**) and the linearity response range of the biosensor in the presence of 0.16–1.5 nM of target sequence

Table 1 The comparison of the sensitivity of reported fluorescence-based methods for the detection of nucleic acid and gene sequences

No.	Method	LOD	Ref. No
1	Based on biocatalytic signal materials and the biotin-streptavidin system	0.033 Fm (S/N = 3)	[17]
2	Integration of gold nanoparticles (AuNPs) and graphene quantum dots (GQDs)	1 nM	[18]
3	Based on gold nanoparticles (AuNPs) and magnetic nanoparticles	2.34 ng/mL	[19]
4	Based on GNPs and MNPs, which The GNPs works as a signal reporter, and the MNPs act as a separator	1.2 ng/ml	[20]
5	An electrochemical method including The ssDNA\AuNP\ctDNA-GCE for detection of complementary and noncomplementary sequences of the SRY gene by the DPV technique	0.5–30.0 ppm	[21]
6	A ratiometric fluorescence biosensor consists of carbon dots and 3-mercapropionic acid-coated cadmium telluride (CdTe) quantum dots	1 nM	[22]

sensitivity for detection of SRY sequence in cell-free fetal DNA. It suggests a simple and cheap method for noninvasive detection of fetal sex by using the mother's blood sample in early stage of pregnancy.

The performance of this method was compared with other reported fluorescence-based biosensors (Table 1). The detection limit of our work, except for Chen et al. [17] compared with methods (Shi et al., 2015; Narmani et al., 2018; Amini et al., 2017; Mazloum-Ardakani et al. 2012; Liang et al. 2017) is better and more sufficient [18–22]. Also, to be cheap, easy, time-consuming are other advantages of the method.

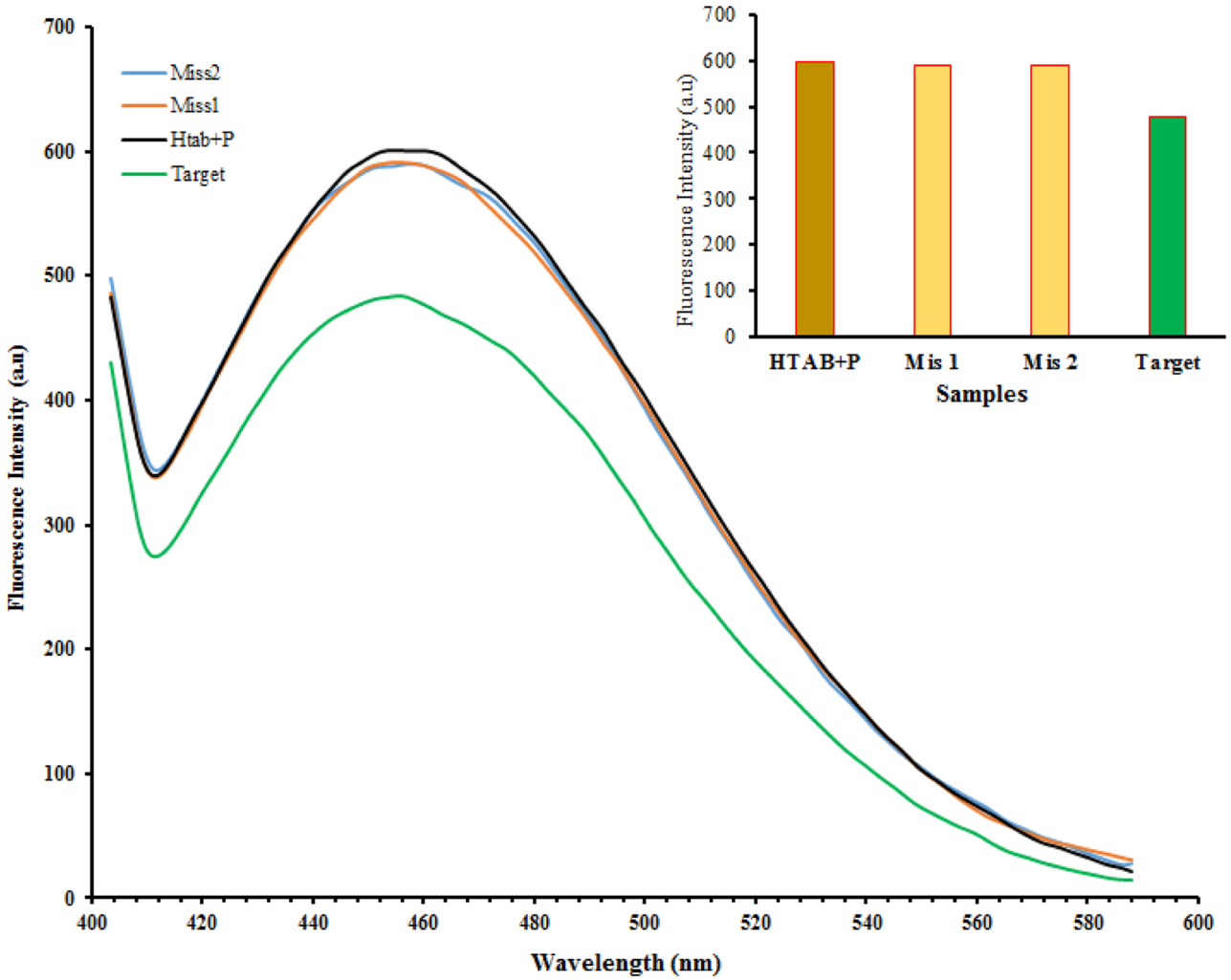


Fig. 8 The selectivity of nanobiosensor for the target sequence

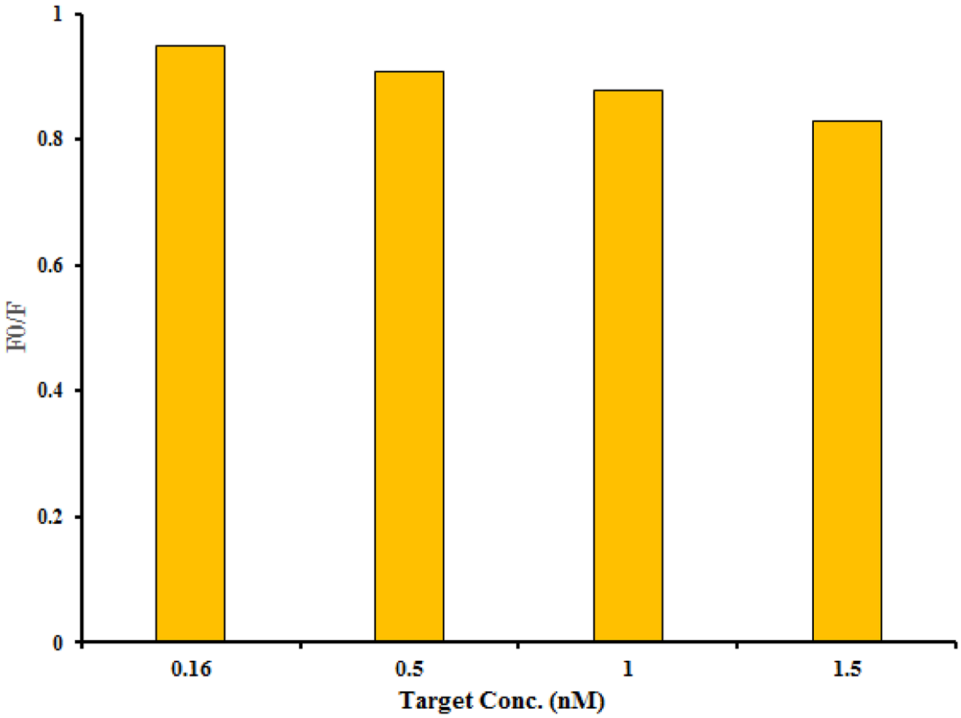


Fig. 9 The decrease of fluorescence intensity with the increase of target concentration in serum media

The Selectivity Assay

Non-complementary nucleotide sequences were used to evaluate the selectivity of the biosensor. As shown in Fig. 8, it can significantly detect and discriminate the target sequence from others. The results showed that the presence of a target sequence is exactly necessary to reduce the fluorescence emission intensity of r-GQD@HTAB. No emission reduction is observed in the without and in presence of non-target sequences. HTAB is more inclined to double-stranded DNA, separates from the r-GQD surface, and is connected to double-stranded DNA, and then it will see a decrease in fluorescence intensity.

Real sample Assay

To propose a clinical utility for the nanobiosensor in sex determination by cffDNA from the mother's blood, it was detected target sequence with different concentrations in blood serum. As shown in Fig. 9, the biosensor could operate for detecting the target and discrimination in a real biological sample similar to a buffer environment, as well.

Conclusion

The design and fabrication of biosensors that can measure and evaluate a specific analyte with low cost, fast and simplicity, and without the need for complex tools is one of the most important issues of clinical scientists. There are several invasive and non-invasive techniques, including amniocentesis and chorionic villus sampling to determine the sex of the fetus before birth. Each of which is performed only during a specific period of pregnancy. Due to concerns about fetal health, it is very important that sex determination techniques are non-invasive and it can be performed, for example, in the early stage using non-invasive specimens such as cffDNA, stool, hair, or various organs that are easy to separate.

The use of nanoparticles such as GQDs in the detection of gene sequences is one of the most popular strategies in detection techniques today. There is very little work has been reported to date on detection by cffDNA as biomarker in fetal medicine. In this study, a novel biosensor was designed and fabricated using r-GQD@HTAB fluorescence properties, which was able to provide us a signal off nanobiosensor with a very high diagnostic limit (LOD = 0.082 nM) for determining the sex of the fetus using the cffDNA mother's blood and SRY gene.

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Authors' Contributions All authors contributed to the study. Material preparation, data collection and experimental works were performed by BOH. The manuscript was written by MR and conceptualization, methodology and visualization were done by MR FF supported a part of conceptualization, methodology and instrumental.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

1. McFarlane L, Truong V, Palmer JS, Wilhelm D (2013) Novel PCR assay for determining the genetic sex of mice. Sex Dev 7(4):207–211
2. Biason-Lauber A (2017) Human sex development: from basic science to clinical practice and back. Pediatr Endocrinol Rev PER 15(1):8–20
3. Odeh M, Grinin V, Kais M, Ophir E, Bornstein J (2009) Sonographic fetal sex determination. Obstet Gynecol Surv 64(1):50–57
4. Lo YD, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW et al (1997) Presence of fetal DNA in maternal plasma and serum. The lancet 350(9076):485–487
5. Lu X, Cui M, Yi Q (2020) Detection of mutant genes with different types of biosensor methods. TrAC Trends Anal Chem 126:115860
6. Farzin MA, Abdoos H (2020) A critical review on quantum dots: from synthesis toward applications in electrochemical biosensors for determination of disease-related biomolecules. Talanta. p 121828
7. Mao L, Liu X, Liu M, Huang L, Xu D, Jiang R et al (2017) Surface grafting of zwitterionic polymers onto dye doped AIE-active luminescent silica nanoparticles through surface-initiated ATRP for biological imaging applications. Appl Surf Sci 419:188–196
8. Walther BK, Dinu CZ, Guldi DM, Sergeyev VG, Creager SE, Cooke JP, et al. (2020) Nanobiosensing with graphene and carbon quantum dots: recent advances. Mater Today
9. Takalkar S, Baryeh K, Liu G (2017) Fluorescent carbon nanoparticle-based lateral flow biosensor for ultrasensitive detection of DNA. Biosens Bioelectron 98:147–154
10. Zhang X, Wei C, Li Y, Yu D (2019) Shining luminescent graphene quantum dots: synthesis, physicochemical properties, and biomedical applications. TrAC, Trends Anal Chem 116:109–121
11. Meng L, Xiao K, Zhang X, Du C, Chen J (2020) A novel signal-off photoelectrochemical biosensor for M. SssI MTase activity assay based on GQDs@ ZIF-8 polyhedra as signal quencher. Biosens Bioelectron 150:111861
12. Zhang R, Sun J, Ji J, Pi F, Xiao Y, Zhang Y et al (2019) A novel "OFF-ON" biosensor based on nanosurface energy transfer between gold nanocrosses and graphene quantum dots for intracellular ATP sensing and tracking. Sens Actuators, B Chem 282:910–916
13. Lin L, Rong M, Luo F, Chen D, Wang Y, Chen X (2014) Luminescent graphene quantum dots as new fluorescent materials for environmental and biological applications. TrAC, Trends Anal Chem 54:83–102
14. Fernando MR, Jiang C, Krzyzanowski GD, Somer-Shely T, Ryan WL (2018) A novel approach to stabilize fetal cell-free DNA fraction in maternal blood samples for extended period of time. PloS one 13(12):e0208508
15. Barrett AN, Xiong L, Tan TZ, Advani HV, Hua R, Laureano-Asibal C et al (2017) Measurement of fetal fraction in cell-free DNA from maternal plasma using a panel of insertion/deletion polymorphisms. PloS one 12(10):e0186771
16. Mirtavoos-Mahyari H, Ghafouri-Fard S, Khosravi A, Motevaseli E, Esfahani-Monfared Z, Seifi S et al (2019) Circulating free DNA concentration as a marker of disease recurrence and metastatic potential in lung cancer. Clin Transl Med 8(1):14
17. Chen J, Yu C, Zhao Y, Niu Y, Zhang L, Yu Y et al (2017) A novel non-invasive detection method for the FGFR3 gene mutation in maternal plasma for a fetal achondroplasia diagnosis based on signal amplification by hemin-MOFs/PtNPs. Biosens Bioelectron 91:892–899
18. Shi J, Chan C, Pang Y, Ye W, Tian F, Lyu J et al (2015) A fluorescence resonance energy transfer (FRET) biosensor based on graphene quantum dots (GQDs) and gold nanoparticles (AuNPs) for the detection of mecA gene sequence of Staphylococcus aureus. Biosens Bioelectron 67:595–600
19. Narmani A, Kamali M, Amini B, Kooshki H, Amini A, Hasani L (2018) Highly sensitive and accurate detection of Vibrio cholera O1 OmpW gene by fluorescence DNA biosensor based on gold and magnetic nanoparticles. Process Biochem 65:46–54
20. Amini B, Kamali M, Salouti M, Yaghmaei P (2017) Fluorescence bio-barcode DNA assay based on gold and magnetic nanoparticles for detection of Exotoxin A gene sequence. Biosens Bioelectron 92:679–686
21. Mazloum-Ardakani M, Heidari M, Naderi M, Sheikh-Mohseni M (2012) Detection of amplified SRY gene by a novel electrochemical biosensor based on gold nanoparticles. Scientia Iranica 19(3):913–918
22. Liang S-S, Qi L, Zhang R-L, Jin M, Zhang Z-Q (2017) Ratiometric fluorescence biosensor based on CdTe quantum and carbon dots for double strand DNA detection. Sens Actuators, B Chem 244:585–590