FLUORESCENCE DETECTION OF MELAMINE BASE ON FUNCTIONALIZED GRAPHENE OXIDE

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Abstract. In this study, we report a new strategy to detect melamine. We present a sensitive and selective fluorescent aptasensor for detection melamine based on graphene oxide (GO) and SYBR Green I. Our strategy utilizes the efficient quenching ability of GO and the different interaction intensity of aptamer, aptamer/melamine complex with GO which directly induces the fluorescence intensity change. The results of experiment showed that, with the reaction mixture consisted of 1×NEBuffer 2, 80 nM T_{55}, 2×SG and different concentration of melamine, the fluorescence of SYBR Green I decreases as the concentration of melamine decreasing. In addition, some conditions and working curve of melamine are established with the concentration of range from 1 nM to 200 nM. Linear regression analysis of detection data yielded the following equation: y = 176.66 + 1.68 x, where y and x denoted the fluorescence peak intensity and melamine concentration, respectively. The peak intensity showed a linear correlation to the concentration of melamine in the range of 10 to 200 nM. The result further demonstrated that the fluorescence recovery was attributed to the formation of between double-stranded (dsDNA) structure by the specific interaction dsDNA and SYBR Green I (SG). Therefore, the GO-based biosensing platform is feasible to be used to selecting for detection melamine. This method provides a simple, rapid and high-throughput method for detection melamine and it could be widely applied to detect small molecules, other proteins and DNAs with specific designed oligonucleotides because of its excellent sequence-independent property.

Keywords. Melamine; fluorescence; graphene oxide; DNA.

1 INTRODUCTION

Melamine [1, 3, 5-Triazine-2, 4, 6-triamine] (Fig.1) is a chemical compound used broadly in the synthesis of melamine resins for manufacturing laminates, plastics, coatings, commercial filters, adhesives, dishware, and kitchen ware [1]. Due to its high nitrogen level (66% by mass) and low cost, melamine was abused to increase the apparent protein level measured by analysis of the total nitrogen content in food [2]. However, melamine is a toxic compound to both animals and human beings, and is connected to various diseases, such as kidney stones and bladder cancer [3, 4]. In addition, melamine is an industrial chemical in the production of melamine resins. It has low oral acute toxicity but chronic administration of high concentrations can induce renal pathology [5]. In March of 2007, pet food ingredients contaminated by melamine and its analogues resulted in a major outbreak of renal disease and associated deaths in cats and dogs in the USA [6]. In September of 2008, high concentration of melamine was reported in contaminated Chinese infant formula. In December of 2008, World Health Organization (WHO) reviewed the latest melamine contamination event of China. More than 51,900 infants and young children in China were hospitalized for urinary problems, possible renal tube blockages and possible kidney stones related to the consumption of melamine contaminated infant formula and related dairy products. Six deaths among infants have been confirmed in mainland China. Levels of melamine in dairy products (including infant formula) ranged from 0.09 to 6196.61mg kg^{-1} [7]. It is presumed that melamine was deliberately added to increase the measured nitrogen content of diluted dairy products according to Kjeldahl testing [8]. The maximum residue levels for melamine in infant formula powder and the other dairy products are 1 and 2.5mgkg^{-1}, respectively, regulated by Chinese government after the

powdered milk scandal. Tolerable daily intake (TDI) is 0.063mg per kg of body weight per day recommended by the US FDA on 3 October 2008 (and updated 28 November) for food and food ingredients other than infant formula [9].

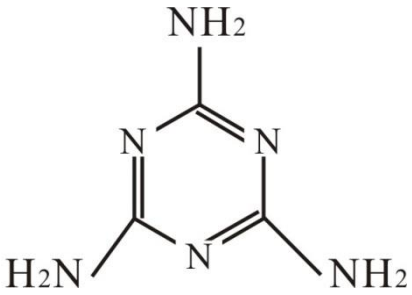


Fig. 1 Structure of melamine

To protect the development of dairy products and the people's safety, it is extremely important and necessary to monitor the amount of melamine in the food and fodders. Many methods for detecting melamine have been established, including capillary zone electrophoresis [10], fluorescence [11], gas chromatography mass spectrometry (GC-MS) [12, 13], reverse phase high performance liquid chromatography (RP-HPLC) [14], and liquid chromatography mass spectrometry (HPLC-MS) [15]. Despite of the success, such methods are known to have some drawbacks operational attributes such as involving high cost, time-consuming and low sensitivity. Thus, it is very necessary to develop a simple and convenient method for the efficient detection of melamine in food. Recently, several new techniques have been studied as: S. Han and coworkers [16] have used oligonucleotide stabilized silver nanoclusters (DNA–AgNCs) and demonstrated that DNA–AgNCs is an alternative probe for the determination of melamine. The method is based on the fluorescence turn on of DNA–AgNCs by melamine. This method is sensitive and selective, and is successfully used for the detection of melamine in milk. H. Huang at al [17] was developed technique label-free and labeled gold nanoparticles to detect melamine in milk. These methods show several analytical advantages such as high sensitivity, selectivity rapid, no expensive and complicated instruments, making on-site and real-time melamine sensing possible. However, the functional legend in this research work was laboratory synthesized, which making the application of this method limited. A variety of strategies have been developed to overcome these shortcomings, especially the introduction of aptamers.

Aptamer for melamine was utilized as molecular recognition agent to build a new sensing platform for detection of melamine. Aptamers are single strand DNA that can bind target molecule with high specificity and strong binding affinity [18]. Aptamers have received tremendous attention in the biosensor applications in recent years, because of their unprecedented advantages such as simple synthesis, easy labeling, long-term stability and excellent target recognition properties [19]. Recently, melamine detection utilizing modified gold nanoparticles (AuNPs) by color changes of the solution has been invented, in which a thiol-functionalized cyanuric acid derivative is labeled on the AuNPs [20-22]. Inspired by the knowledge that folded DNA structure and hybrid duplex are resistant to the adsorption onto basal plane of graphene oxide which has attracted great attention in the past several years we postulated that folded aptamer induced by binding to melamine was also resistant to adsorption onto the surface of graphene oxide. Therefore, we designed and developed new highly selective and sensitive aptamer-based sensors for determination melamine that are simple and cost-effective.

Since the discovery of graphene by Professor Andre Geim in 2004 (Nobel prize winner for 2010) and the first application of graphene based advanced materials [23], this material has been utilized in various fields and the related references have expanded dramatically. In the past few years, graphene has been widely applied in nanoelectronics [24], nanocomposites [25], biosensors [26], and hydrogen storage [27]. By combining graphene oxide with aptamer modified in single end, the major advantage is that it eliminates expensive dual labeling of aptamer in comparison to conventional molecular beacon. It is very important to note that graphene oxide based sensors have to meet one prerequisite that the analyzed target

does not nonspecifically adsorb onto the surface under the experimental conditions, otherwise the sensing performance is dramatically degraded or the sensor fails to function. In addition, GO is known to efficiently quench the fluorescence of organic dyes through long-range nanoscale energy transfer. The GO was reported to interact strongly with nucleotides of single-stranded DNA (ssDNA) through π-stacking interaction between the ring structures in the nucleobases and the hexagonal cells of GO, whereas double-stranded DNA cannot be stably adsorbed onto the GO surface because of efficient shielding of nucleobases within the negatively charged phosphate backbone of dsDNA. Based on this property of the preferential binding of GO to ssDNA over dsDNA, combined with the highly efficient quenching effect, a series of convenient and versatile strategies have been explored for the detection of DNA [28], metal ions [29], small molecules [30], protein [31], the screening of aptamers [32], and even in situ cellular imaging [33].

In this study, we report a new strategy to detect melamine. We present a sensitive and selective fluorescent aptasensor for detection melamine based on GO and SYBR Green I. Our strategy utilizes the efficient quenching ability of GO and the different interaction intensity of aptamer, aptamer/melamine complex with GO which directly induces the fluorescence intensity change. Scheme 1 describes the mechanism of the biosensing platform for selecting melamine. Because of the π-π stacking of DNA bases with GO, the aptamer signal probe can bind closely to the surface of GO and result in highly efficient quenching of fluorescence of the SYBR Green I. When the melamine are added, the aptamer signal probe is combined with melamine, which results in the releasing the aptamer probe from GO surface. Then, this aptamer is completed with SYBR Green I and the fluorescence recovery is observed.

2 EXPERIMENTAL

2.1. Apparatuses and reagents

All fluorescence measurements were carried out on an F 7000 spectrofluorometer (Hitachi, Japan) equipped with a thermostat accurate to 0.1°C. Slits were set at 5 nm for the excitation and 5 nm for the emission. The emission spectra were obtained by exciting the samples at 490 nm and scanning the emission from 500 to 600 nm with scan speed 240nm/min and PMT voltage 900 V. All samples were incubated at 37°C then measured in curvet 100µL. The time curves of the fluorescence intensity of solutions were recorded.

SYBR Green I (SG) was obtained from Sigma-Aldrich. Co (St. Louis, MO). The buffer 10×NEBuffer 2 (500 mM NaCl, 100 mM Tris-HCl, 100 mM $MgCl_2$, 10 mM Dithiothreitol, pH 7.9) was purchased from New England Biolabs Inc. Graphite powder, melamine, sulfuric acid, potassium persulfate, phosphorus pentoxide, hydrogen peroxide, and potassium permanganate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) which were of analytical grade and were used without further purification. Deionized water was obtained through the Nanopure Infinity ultrapure water system (with an electric resistance > 18.3 MΩ). All the oligonucleotides used in this work were synthesized from Takara Biotechnology Co. Ltd. (Dalian, China). The sequences of these oligonucleotides were shown in table 1.

Table 1. Synthesized Oligonucleotides (5’ → 3’) used in the Experiments.

Probe name	Probe symbol	Probe structure
Probe 1	G35	GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
Probe 2	C35	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
Probe 3	A35	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Probe 4	T55	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT

2.2. Synthesis of graphene oxide

Graphite oxide was synthesized from natural graphitic powder according to Hummer's method [31, 34] with some modification. In detail, graphite powder (3.0 g) was treated with an 80°C mixture solution containing concentrated H_2SO_4 (12 mL) in a 1000 mL flask, $K_2S_2O_8$ (2.5g), and P_2O_5 (2.5 g). The mixture was kept at 80°C for 4h using oil bath. Successively, the mixture was diluted with 0.5L of de-ionized water after being cooled to room temperature, and left overnight. Then, the mixture was filtered and washed with de-ionized water to remove the residual acid and dried under ambient condition overnight. The initial product was re-dispersed in to 0°C concentrated H_2SO_4 (120mL). Then, $KMnO_4$ (15 g) was gradually added to the mixture in an ice bath and thoroughly mixed. Successively, the mixture was stirred at 35°C for 2h, followed by dilution with de-ionized water (250mL). After continuously stirring for another 2 h, additional 0.7L of de-ionized water and 20mL of 30% H_2O_2 was added to the mixture drop by drop by turns, in which the color of mixture changed in to brilliant yellow. The mixture was filtered and washed with 1L of 10% HCl aqueous solution aqueous solution and 1 L of de-ionized water, to remove metal ions, and the acid, respectively. The resulting solid was dried in air. Finally, as synthesized product was purified by dialysis for one week to remove the remaining metal species, and then dispersed in water under sonication for 5h. The resulting dispersion was subjected to 10 min of centrifugation at 3000 rpm to remove un-exfoliated GO.

2.3. Verify the specificity of the sensor

To verify the specificity of the sensor, we performed at various different oligonucleotides as follow: The reaction mixture contained 1×NEBuffer 2, 5×SG, 200 nM melamine and 50 nM DNA for each (G35, C35, A35, and T55). This mixture was mixed and incubated in water bath at 37°C for 30 min. After reaction, the mixture was added 500 μg/mL GO and incubated for 10 min. The last, this mixture was measured by using an F-7000 spectrofluorometer.

2.4. Optimization of assay conditions

2.4.1. Optimization of SG concentration

To optimize the concentration of SG, we performed at various concentration of SG (1×, 2×, 3×, 4×, 5×), respectively. The reaction mixture consisted of 1×NEBuffer 2, 200 nM melamine and 50 nM DNA (T55, probe 4). The mixture was stained by SYBR Green I with different concentration and incubated at 37 °C for 30 minutes, continue to be added to the mixture 500 μg/mL GO. After, this solution was incubated for 10 min at 37 °C and measured by using an F-7000 spectrofluorometer. We performed background same as the above, but without melamine.

2.4.2. Optimization of GO concentration

To optimize the concentration of GO, we performed at various concentration of GO (50, 100, 200, 300, 500, 800 and 1000 μg/mL), respectively. The reaction mixture consisted of 1×NEBuffer 2, 200 nM melamine, 5×SG and 50 nM DNA (T55, probe 4). The mixture was incubated at 37 °C for 30 minutes, continue to be added to the mixture different concentration of GO. After, this solution was incubated for 10 min at 37 °C and measured by using an F-7000 spectrofluorometer. We performed background same as the above, but without melamine.

2.4.3. Optimization of T55 concentration

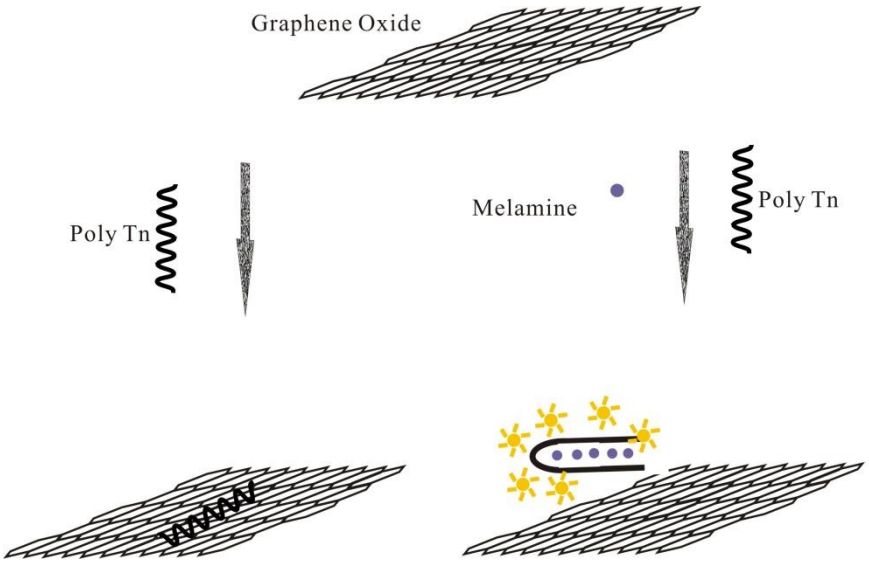
To optimize the concentration of oligonucleotide (T55, probe 4), we performed at various concentration of T55 (10, 20, 50, 80, 100, and 200 nM), respectively. The reaction mixture consisted of 1×NEBuffer 2, 200 nM melamine, 5×SG and different concentration of DNA (T55, probe 4). The mixture was incubated at 37 °C for 30 minutes, continue to be added to the mixture 500 μg/mL GO. After, this solution was incubated for 10 min at 37 °C and measured by using an F-7000 spectrofluorometer. We performed background same as the above, but without melamine.

2.5. Detection of melamine

According to optimization of conditions as above, we performed at various concentration of melamine from 1 to 200 nM. The reaction mixture consisted of 1×NEBuffer 2, 80 nM probe 4, 2×SG and different concentration of melamine. The mixture was incubated at 37 °C for 30 minutes, continue to be added to the mixture 200 μg/mL GO. After, this solution was incubated for 10 min at 37 °C and measured by using an F-7000 spectrofluorometer. Each experiment was repeated three times.

3 RESULT AND DISSCUSION

3.1. Probe design and analytical principle



Scheme 1. Schematic representation of the sensing processes

The principle of method is shown in Scheme 1. In this study, we designed an aptamer selection for melamine that its structure is a poly-T nucleotide chain (T55). The biosensing platform is constructed according to the non-covalent assembly of aptamer (T55) on graphene which is induced by π-π stacking of DNA bases on graphene. In the absence of melamine, shows weak fluorescence owing to the strong adsorption of T55 on GO surface via the π-π stacking attraction and super fluorescence quenching ability of GO, because of poly-T nucleotide chain corresponds to a single stranded DNA that binding force of SG with single-stranded DNA is relatively weak. Upon the addition of melamine, competitive binding of melamine and GO with T55 causes the release of T55 from GO surface, allowing fluorescence-signal enhancement. The reason is explained as follows: when the presence of melamine can induce conformational changes of poly-T nucleotide chain, because melamine can form hydrogen bonds with T nucleotide and poly-T nucleotide chain corresponds double-stranded DNA. Therefore, melamine detection could be easily realized by monitoring the change of fluorescence signal.

3.2. Signal amplification of the sensing system

In order to evaluate the amplification function of the proposed sensing system, we surveyed many different oligonucleotides chain: poly-G (G35), poly-C (C35), poly-A (A35) and poly-T (T55) nucleotide chain. The experimental results were shown in Fig. 2. From the results, only the poly-T nucleotide chain has strong fluorescence signal (I. F. ~ 580 a. u.). In contrast, other poly nucleotide have weaker fluorescence signal (I. F. ~ 160 a. u.). These results confirm that this poly-T nucleotide can enable significant signal amplification for melamine detection.

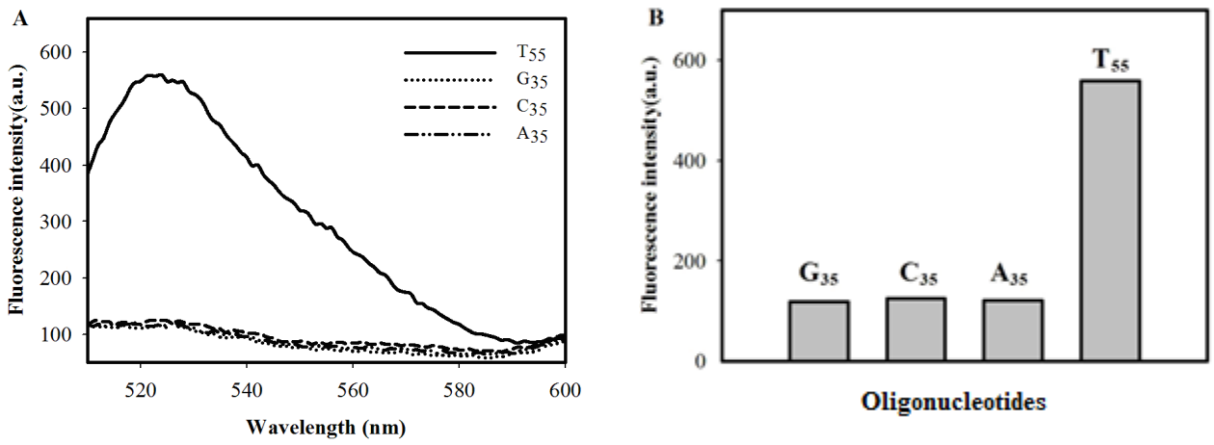


Fig. 2 (A) Fluorescence emission spectra of system at different oligonucleotides, (B) The plot of relative fluorescence intensity vs different oligonucleotides, with in the presence 5xSG, 500 µg/mL GO and 200 nM melamine.

3.3. Optimization of analytical conditions

In order to obtain an optimal analytical condition and achieve a high signal-to-noise ratio, the effects of GO, SG and DNA concentrations have been investigated by fixing them together and change one element of them, respectively.

3.3.1 Optimization of SG concentration

In Fig. 3, we can see that the F/F0 ratio of the sensing system increased significantly when the concentrations of SG ranging from 1X to 2X. However, when the concentration of SG was higher than 2X, the F/F0 ratio decreased with a further increasing concentration of SG, which might be ascribed to the excessive quenching effect of the GO at a high concentration on the cleavage-produced fluorophore. Therefore, we chose 2X SG as the optimum analytical condition.

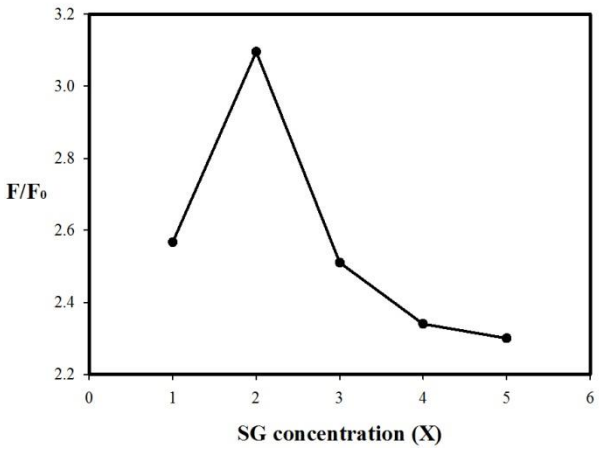


Fig. 3 The plot of relative fluorescence intensity vs the concentration of SG, relative fluorescence intensity is calculated by F/F0, where F0 and F represent the fluorescence intensity of the SG and probe 4/GO complex before and after incubation with 200 nM melamine, respectively. The concentration of probe 4 was 50 nM. The excitation wavelength was 490 nm

3.3.2. Optimization of GO concentration

In Fig. 4, we can see that the F/F0 ratio of the sensing system increased significantly when the concentrations of GO ranging from 50 to 200 µg/mL. However, when the concentration of GO was higher than 200 µg/mL, the F/F0 ratio decreased with a further increasing concentration of GO, which might be

ascribed to the excessive quenching effect of the GO at a high concentration on the cleavage-produced fluorophore [35]. Therefore, we chose 200 µg/mL GO as the optimum analytical condition.

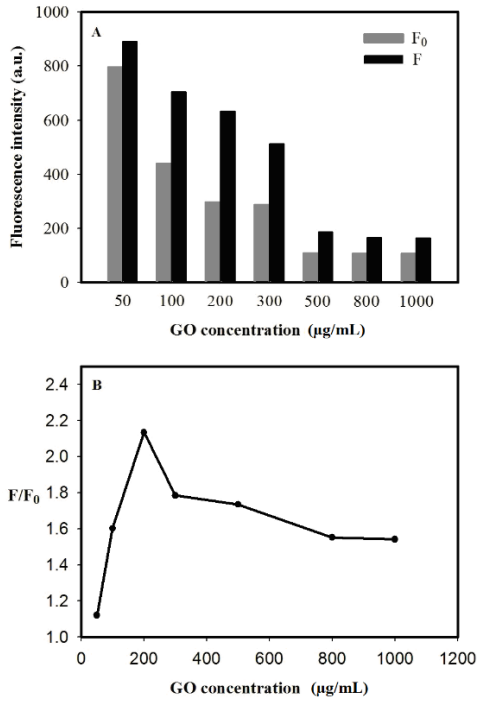


Fig. 4 (A) The effect of the concentration of GO on the efficiency of the fluorescence recovery. The black bars (F0) and the gray bars (F) represent the fluorescence intensity of SG, probe 4/GO complex before and after incubation. (B) The plot of relative fluorescence intensity vs the concentration of GO. Relative fluorescence intensity is calculated by F/F0, where F0 and F represent the fluorescence intensity of the SG, probe 4/GO complex before and after incubation. The concentration of probe 4 was 50 nM. The excitation wavelength was 490 nm.

3.3.3. Optimization of T_{55} concentration

Next, the effect of concentration of DNA was further studied and the results were shown in Fig. 5. It is clear that the F/F0 ratio of the sensing system increased with increasing DNA concentration until the concentration reached 80 nM. However, when the concentration exceeded 80 nM, the F/F0 ratio of the sensing system decreased. Taking into account the response sensitivity, 80 nM was used in the final solution.

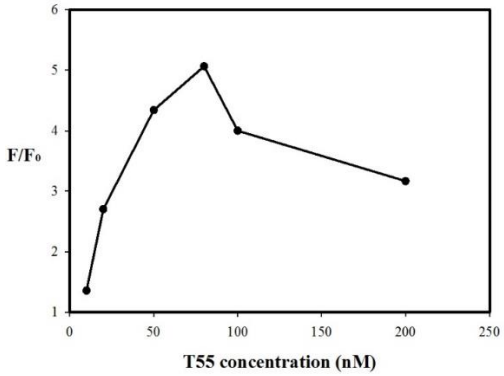


Fig. 5 The plot of relative fluorescence intensity vs the concentration of T55, relative fluorescence intensity is calculated by F/F0, where F0 and F represent the fluorescence intensity of the SG and probe 4/GO complex before and after incubation with 200 nM melamine, respectively. The excitation wavelength was 490 nm

3.4. Detection of melamine

Under the optimal experimental conditions, the relationship between the melamine concentration and fluorescence enhancement was investigated. Fig. 6 showed the fluorescence intensity of SG and probe 4/GO complex after incubation with a series of different concentrations of melamine at 37◦C for 30 min. The fluorescence intensity of the mixture solution continuously increased with the concentration of melamine ranging from 1 nM to 200 nM, indicating that more and more probe 4 formed the dsDNA structure and released from GO surface with the increasing melamine concentration, and the increase of fluorescence intensity can quantitatively reflect the amount of melamine added. Linear regression analysis of detection data yielded the following equation: y = 176.66 + 1.68 x, where y and x denoted the fluorescence peak intensity and melamine concentration, respectively. The peak intensity showed a linear correlation to the concentration of melamine in the range of 10 to 200 nM. The result further demonstrated that the fluorescence recovery was attributed to the formation of dsDNA structure by the specific interaction between dsDNA and SG. Therefore, the GO-based biosensing platform is feasible to be used to selecting for detection melamine.

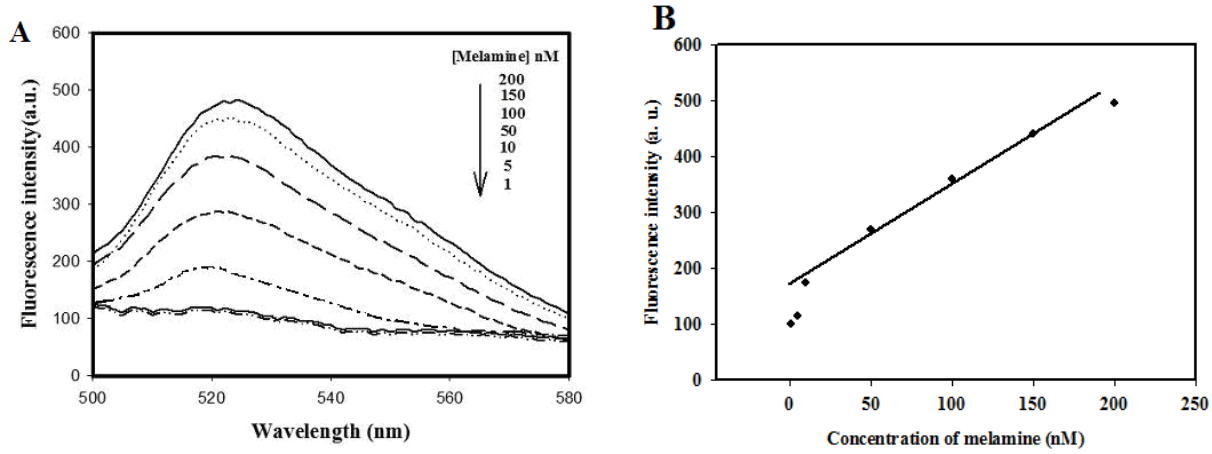


Fig. 6 (A) Typical fluorescence spectral responses of the biosensing strategy to melamine of varying concentrations, (B) Linear relationship intensity fluorescence and the concentration of melamine.

4 CONCLUSION

In this study, we have developed a strategy for detection melamine by using GO as the fluorescence quencher. Through π-stacking interaction between the ring structure in the nucleobases and the hexagonal cells of GO, probe 4 adsorbed onto the surface of GO, and the fluorescence of the SG and DNA was quenched. When melamine were introduced, the probe 4 folded to form dsDNA structure, which led to the releasing of probe 4 from the surface of GO, and that binding to SG. So, the fluorescence intensity increased. This method provides a simple, rapid and high-throughput method for detection melamine and it could be widely applied to detect small molecules, other proteins and DNAs with specific designed oligonucleotides because of its excellent sequence-independent property.

ACKNOWLEDGMENT

The authors greatly appreciate the financial support by the National Natural Science Foundation of China (no. 21175040).

REFERENCES

[1] C. W. Kim, J. W. Yun, I. H. Bae, et al. Determination of Spatial Distribution of Melamine-Cyanuric Acid Crystals in Rat Kidney Tissue by Histology and Imaging Matrix-Assisted Laser Desorption/Ionization Quadrupole Time-of-Flight Mass Spectrometry. *Chemical Research Toxicology*, 2010, 23, 220–227.

[2] M. C. Chiu. Melamine-tainted-milk product (MTMP) renal stone outbreak in humans. *Hong Kong Medical Journal*, 2008, 14, 424–426.

[3] L.M. Allen, T.V. Briggle, C.D. Pfaffenberger. Absorption and excretion of cyanuric acid in long-distance swimmers. *Drug Metab Rev Journal*, 1982, 13, 499– 516.

[4] C.B. Langman, U. Alon, J. Ingelfinger, et al. A position statement on kidney disease from powdered infant formula-based melamine exposure in Chinese infants. *Pediatric Nephrology Journal*, 2009, 24, 1263–1266.

[5] H. Ogasawara, K. Imaida, H. Ishiwata, et al. Urinary bladder carcinogenesis induced by melamine in F344 male rats: correlation between carcinogenicity and urolith formation. *Carcinogenesis*, 1995, 16, 2773 – 2777.

[6] M.S. Filigenzi, B. Puschner, L.S. Aston, R.H. Poppenga. Diagnostic Determination of Melamine and Related Compounds in Kidney Tissue by Liquid Chromatography/Tandem Mass Spectrometry. *Journal of Agricultural and Food Chemistry*, 2008, 56, 7593 – 7599.

[7] Held in collaboration with FAO and supported by Health Canada Ottawa, Canada, 1–4 December 2008, Melamine-contamination event, China: Expert meeting to review toxicological aspects of melamine and cyanuric acid (available in website http://www.who.int/foodsafety/fs_management/infosan_events/en/)

[8] E.Y.Y. Chan, S.M. Griffiths, C.W. Chan. Public-health risks of melamine in milk products. *Lancet*, 2008, 372, 1444 – 1445.

[9] J. Litzau, G. Mercer, K. Mulligan, GC-MS Screen for the Presence of Melamine, Ammeline, Ammelide and Cyanuric Acid (version 2.1), (available in website http://www.fda.gov/AboutFDA/CentersOffices/OfficeofFoods/CVM/WhatWeDo/ucm134742.htm)

[10] J. Xia, N. Y. Zhou, Y. J. Liu, et al. Simultaneous determination of melamine and related compounds by capillary zone electrophoresis. *Food Control*, 2010, 21, 912–918.

[11] Y. Y. Zhou, J. Yang, M. Liu, et al. A novel fluorometric determination of melamine using cucurbit[7]uril. *Journal of Luminescence*, 2010, 130, 817–820.

[12] J. Li, H. Y. Qia, Y. P. Shia. Determination of melamine residues in milk products by zirconia hollow fiber sorptive microextraction and gas chromatography–mass spectrometry. *Journal of Chromatography A*, 2009, 1216, 5467–5471.

[13] X. M. Xua, Y. P. Ren, Y. Zhu, et al. Direct determination of melamine in dairy products by gas chromatography/mass spectrometry with coupled column separation. *Analytica Chimica Acta*, 2009, 650, 39–43.

[14] H. W. Sun, L. X. Wang, L. A. F Ai, et al. A sensitive and validated method for determination of melamine residue in liquid milk by reversed phase high-performance liquid chromatography with solid-phase extraction. *Food Control*, 2010, 21, 686–691.

[15] S.A. Tittlemier, et al. Analysis of melamine in various milk-containing products by LC–MS/MS. Ottawa, Ontario, *Health Canada*, 2008 (NCR_WI.0013).

[16] S. Han, S. U. Zhu, Z. Y. Liu, et al. Oligonucleotide-stabilized fluorescent silver nanoclusters for turn-on detection of melamine. *Biosensors and Bioelectronics*, 2012, 36, 267–270.

[17] H. Huang, L. Li, G. H. Zhou, et al . Visual detection of melamine in milk samples based on label-free and labeled gold nanoparticles. *Talanta*, 2011, 85, 1013– 1019.

[18] A.D. Ellington, J.W. Szostak, In vitro selection of RNA molecules that bind specific ligands. *Nature*, 1990, 346, 818–822.

[19] I. German, D. D. Buchanan, and R. T. Kennedy. Aptamers as Ligands in Affinity Probe Capillary Electrophoresis. *Analytical Chemistry*, 1998, 70, 4540-4545.

[20] K. L. Ai, Y. L. Liu, and L.H. Lu. Hydrogen-Bonding Recognition-Induced Color Change of Gold Nanoparticles for Visual Detection of Melamine in Raw Milk and Infant Formula. *Journal of the American Chemical Society*, 2009, 131, 9496–9497.

[21] H. Ping, M. W. Zhang, H. K. Li, et al. Visual detection of melamine in raw milk by label-free silver nanoparticles. *Food Control*, 2012, 23, 191 – 197.

[22] L. Li, B. X. Li, D. Cheng, L.H. Mao. Visual detection of melamine in raw milk using gold nanoparticles as colorimetric probe. *Food Chemistry*, 2010, 122, 895–900.

[23] K. S. Novoselov, A. K. Geim, S. V. Morozov, et al. Two-dimensional gas of massless Dirac fermions in Grapheme. *Nature*, 2005, 438, 197–200.

[24] A. Savchenko. Transforming Graphene. *Science*, 2009, 323, 589–590.

[25] S. Stankovich, D. A. Dikin, G. H. B. Dommett, et al. Graphene-based composite materials. *Nature*, 2006, 442, 282–286.

[26] Y. Wan, Y. Wang, J. J. Wu, and D. Zhang. Graphene Oxide Sheet-Mediated Silver Enhancement for Application to Electrochemical Biosensors. *Analytical Chemistry*, 2010, 83, 648–653.

[27] Y.C. Si, E. T. Samulski. Exfoliated Graphene Separated by Platinum Nanoparticles. *Chemistry of Materials*, 2008, 20, 6792–6797.

[28] C. H. Lu, H. H. Yang, C. L. Zhu, et al. A Graphene Platform for Sensing Biomolecules. *Angew Angewandte Chemie International Edition*, 2009, 48, 4785–4787.

[29] Y.Q. Wen, F.F. Xing, S.J. He, et al. A graphene-based fluorescent nanoprobe for silver (I) ions detection by using graphene oxide and a silver-specific oligonucleotide. *Chemical Communications*, 2010, 46, 2596–2598.

[30] Y. Wang, Z. H. Li, D. H. Hu, et all. Aptamer/Graphene Oxide Nanocomplex for in Situ Molecular Probing in Living Cells. *Journal of the American Chemical Society*, 2010, 132, 9274–9276.

[31] H. X. Chang, L. H Tang, Y. Wang, et al. Graphene Fluorescence Resonance Energy Transfer Aptasensor for the Thrombin Detection. *Analytical Chemistry*, 2010, 82, 2341–2346.

[32] J. W. Park, R. Tatavarty, D. W. Kim, et al. Immobilization-free screening of aptamers assisted by graphene oxide. *Chemical Communications*, 2012, 48, 2071-2073.

[33] L.M. Zhang, J.G. Xia, Q.H. Zhao, et al. Functional graphene oxide as nanocarriers for controlled loading and targeted delivery of mixed anticancer drugs. *Small*, 2010, 6, 537-544.

[34] X.J. Xing, X. G. Liu, Y. He, et al. Graphene oxide based fluorescent aptasensor for adenosine deaminase detection using adenosine as the substrate. *Biosensors and Bioelectronics*, 2012, 37, 61–67.

[35] X. H. Zhao, Q. J. Ma, X. X. Wu, X. Zhu. Graphene oxide-based biosensor for sensitive fluorescence detection of DNA based on exonuclease III-aided signal amplification. *Analytica Chimica Acta*, 2012, 727, 67– 70.

XÁC ĐỊNH MELAMIN BẰNG PHƯƠNG PHÁP HUỲNH QUANG DỰA TRÊN TÍNH CHẤT CỦA GRAPHEN OXIT

Tóm tắt. Trong nghiên cứu này, chúng tôi báo cáo một phương pháp mới để xác định melamin. Chúng tôi xây dựng một phương pháp nhạy, chọn lọc melamin dựa trên sự phát huỳnh quang của hệ graphen oxit (GO) và chất nhuộm màu DNA SYBR Green I. Cơ chế phát huỳnh quang được tuân theo quy luật như sau: Khi không có melamin, chuỗi DNA sẽ nằm trên nền GO dưới dạng chuỗi đơn, khi đó tín hiệu huỳnh quang sẽ rất yếu do sự nhuộm màu của SG kém đối với chuỗi DNA đơn. Trong khi có mặt của melamin thì chuỗi DNA sẽ tương tác với chúng và tạo thành chuỗi tương đương chuỗi DNA đôi, khi đó sự nhuộm màu cho chuỗi này sẽ phát huỳnh quang mạnh hơn, vì thế sự phát huỳnh quang của hệ thay đổi theo hàm lượng melamin. Bằng thực nghiệm, chúng tôi đã tối ưu hóa được một số điều kiện quan trọng như nồng độ của GO, SG, T_{55}… và xây dựng được khoảng nồng độ của melamin từ 1 nM tới 200 nM. Đối với phương pháp mà chúng tôi xây dựng với hy vọng có được một phương pháp mới, nhạy và chọn lọc để xác định melamin.

Từ khóa. Melamine; fluorescence; graphene oxide; DNA.

Ngày nhận bài: 14/06/2017

Ngày chấp nhận đăng: 21/11/2017