Hindawi Publishing Corporation
Journal of Sensors
Volume 2016, Article ID 5862929, 6 pages
http://dx.doi.org/10.1155/2016/5862929

Research Article

A Highly Selective Colorimetric Sensor for Cysteine in Water Solution and Bovine Serum Albumin

Xuefang Shang,[1] Jie Yu,[1] Xiaofang Wei,[2] Xin Li,[2] Yaqian Feng,[2] and Xiufang Xu[3]

[1]*Department of Chemistry, Xinxiang Medical University, Xinxiang, Henan 453003, China*
[2]*School of Pharmacy, Xinxiang Medical University, Xinxiang, Henan 453003, China*
[3]*Department of Chemistry, Nankai University, Tianjin 300071, China*

Correspondence should be addressed to Xuefang Shang; xuefangshang@126.com

Received 18 August 2015; Revised 1 November 2015; Accepted 15 November 2015

Academic Editor: Hana Vaisocherova

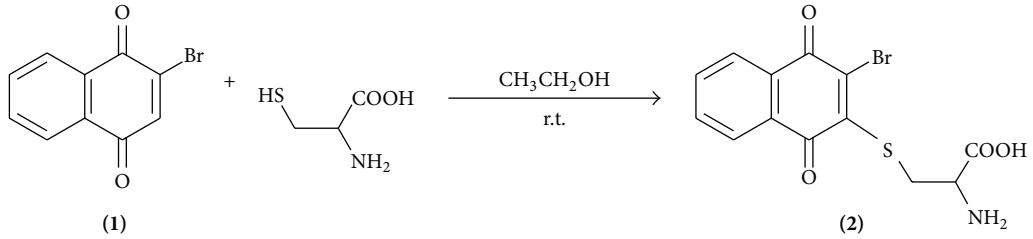
A simple colorimetric sensor, 2-bromonaphthalene-1,4-dione, has been developed for the Cysteine detection. The sensor showed its best performance in a mixture of ethanol and HEPES (5 : 5, v/v) solution at pH of 7.0. The results of UV-vis and fluorescence indicated that 2-bromonaphthalene-1,4-dione was selective and sensitive for Cysteine detection without the interference of other amino acids (Cysteine, Alanine, Arginine, Aspartinie, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Proline, Serine, Threonine, Phenylalanine, Valine, Tryptophan, and Hydroxyproline). 2-Bromonaphthalene-1,4-dione also showed binding ability for Cysteine in bovine serum albumin and could be used as a potential colorimetric sensor among eighteen kinds of natural amino acids. Importantly, the recognition of CySH could be observed by naked eye.

1. Introduction

The assay of amino acids in different food, biological, and chemical samples is of particular importance [1–5]. Cysteine (CySH, 2-amino-3-mercaptopropionic acid), which is one kind of important amino acids, plays a vital role in biological systems and has been widely used in medicine and food chemistry [6, 7]. Slowed growth, hair depigmentation, edema, lethargy, liver damage, muscle and fat loss, skin lesions, and weakness are related to the deficiency of CySH [8, 9]. In addition, hypoglycemic brain damage induced by the increase of CySH level has been studied as an alternative mechanism to excitotoxicity. Therefore, studies focusing on the CySH determination are very important from the biological and pharmacological standpoints and have attracted considerable attentions nowadays [10–15]. In this sense, several methods for its detection and quantification have been reported including UV-vis detection [16], fluorescent detection [17], mass spectrometry identification [18], high performance liquid chromatography (HPLC) [19, 20], Fourier transform infrared (FTIR) detection [21, 22], capillary electrophoresis (CE) [23], and electrochemical methods [24, 25]. However, most of them experienced many difficulties with sample preparation, necessity of molecules derivatization, or lack of sufficient sensitivity, which limit their practical utility [19].

On the other hand, fluorescent methods present the advantages of simplicity and high sensitivity. Recently, a great effort has gone into the development of selective fluorescence sensors. One key problem for CySH detections is the recognition of CySH from other amino acids. Although fluorescent and visual detection methods for free amino acids have been reported, selective recognition of CySH among amino acids is quite limited [10–12]. Huang's group has reported naked-eye sensors for CySH containing Azo dyes or cyanide group. However, the synthesis of sensors is complicated. In this paper, we reported a sensor for CySH employing 2-bromonaphthalene-1,4-dione (**1**) (Scheme 1) under neutral pH with high selectivity and sensitivity and studied its binding ability for Cysteine in bovine serum albumin (BSA).

Chen et al. [26] reported the reaction of 2-methylnaphthalene-1,4-dione with 3-thiopropionic acid under ethanol solvent. According to Chen's research, mild conditions should be chosen to reduce the S_NAr^H reaction velocity and enhance



Scheme 1: Reaction of 2-bromonaphthalene-1,4-dione (**1**) with Cysteine.

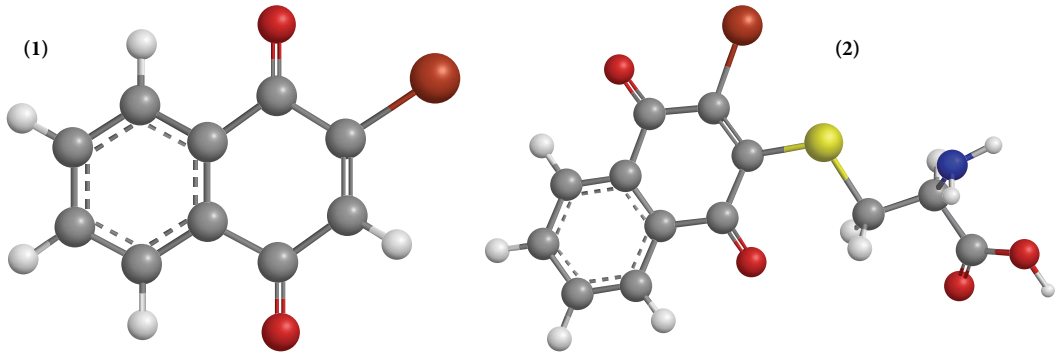


Figure 1: Optimized structures of 2-bromonaphthalene-1,4-dione (**1**) and thiazolidinedione derivative (**2**).

selectivity upon the dependence of steric hindrance on the reaction velocity. The above results indicated 2-methylnaphthalene-1,4-dione could be used to bind some molecules containing HS group. Herein, the recognition of CySH with 2-bromonaphthalene-1,4-dione was investigated in a mixture of ethanol and HEPES (5 : 5, v/v) solution at pH of 7 by UV-vis absorption and fluorescence techniques (Scheme 1).

2. Materials and Methods

Most of the starting materials were obtained commercially and all reagents and solvents used were of analytical grade. All amino acids, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), and bovine serum albumin were purchased from Sigma-Aldrich Chemical Co. (Shanghai, China), stored in a desiccator under vacuum containing self-indicating silica, and used without any further purification. BSA was purchased from Sigma-Aldrich Chemical Co. (Shanghai, China). C, H, and N elemental analyses were made on Vanio-EL (Heraeus, Germany). ^{1}H NMR spectra were recorded on a Varian UNITY Plus-400 MHz Spectrometer (Bruker, Germany). UV-vis spectroscopy titrations were recorded on a Shimadzu UV-2450 Spectrophotometer (Japan) at 298 ± 0.1 K. Fluorescence spectroscopy was performed on a Cary Eclipse Fluorescence Spectrophotometer (Agilent, America) at 298 ± 0.1 K. ESI-MS was performed with a LC-MS apparatus (Agilent, America).

The sensor, 2-bromonaphthalene-1,4-dione, was synthesized according to the previous literature [27]. Naphthoquinone (10 g) was dissolved in glacial acetic acid (200 mL) and the solution cooled to incipient crystallization. The solution was protected from light, and bromine (4.0 mL) was added from a pipet. The mixture was allowed to stand in the dark for four hours, and a stream of carbon dioxide then was passed through for an hour in order to sweep out the excess bromine. Fused sodium acetate (20 g) was added and the mixture was stirred until the salt was dissolved. The solution then was allowed to stand for twenty-four hours at room temperature, time after which it was poured into cold water (2 L). The product was collected and dried and was characterized by ^{1}HNMR, elemental analysis, and ESI-MS. Yield: 87%. ^{1}H NMR (DMSO-d_6): δ 8.91 (s, 1H), 8.37 (d, 2H), 8.21 (dd, 2H). Elemental analysis: Calc. for $C_{10}H_5BrO_2$: C, 50.67; H, 2.13; Found: C, 50.82; H, 2.36. ESI-MS (m/z): 236.1 $(M\text{-H})^-$.

3. Results and Discussion

3.1. Theoretical Investigation. To understand the effect of naphthoquinone derivative on the amino acid binding properties, the geometries of 2-bromonaphthalene-1,4-dione and host-guest complexes (thiazolidinedione, **2**) were optimized (Figure 1) using density functional theory at the B3LYP/3-21G level with Gaussian 03 program [28]. The following UV-vis and fluorescence experiments were determined in solution in which the influence was low and could be ignored. Therefore, the optimization was conducted in atmosphere. According to the theoretical investigation, the total energies of compounds **1** and **2** were −3093.3709 and −3810.3525 a.u., respectively. The total energy of compound **2** was lower than that of compound **1** by 716.98 a.u. which indicated thiazolidinedione (**2**) was more stable than the corresponding compound (**1**) thermodynamically and thus the binding ability of Cysteine was more sensitive.

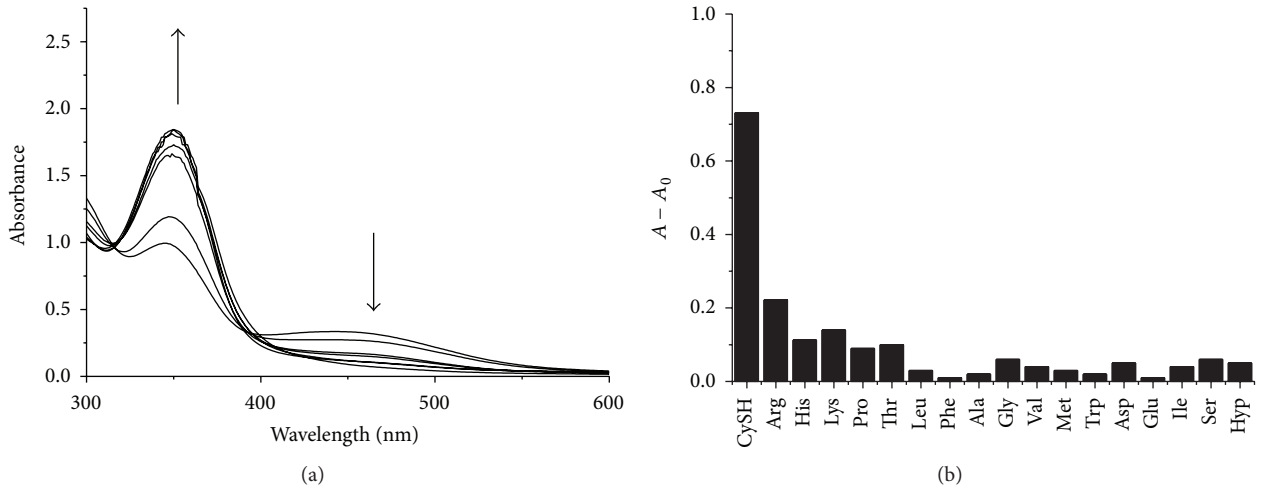


FIGURE 2: (a) UV-vis spectra of 2-bromonaphthalene-1,4-dione (2×10^{-5} mol·L^{-1}) in the presence of different concentration of CySH (0–2 $\times 10^{-5}$ mol·L^{-1}) at 298 K. Condition: in ethanol-HEPES solution (5 : 5, v/v, pH of 7). The spectrum is acquired 5 min after CySH addition. Arrows indicate the direction of increasing CySH concentration. (b) UV-vis spectral changes of sensor upon the additions of various amino acids.

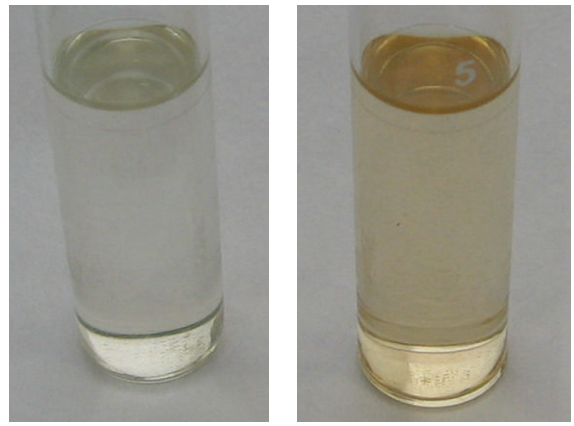


FIGURE 3: The color change of 2-bromonaphthalene-1,4-dione (2×10^{-5} mol·L^{-1}) in ethanol-HEPES solution (5 : 5, v/v, pH of 7) in the presence of 1 equiv. of CySH. (a) Absence of amino acid and (b) CySH.

3.2. UV-vis Spectra. For an excellent chemosensor, high selectivity is a matter of necessity. UV-vis spectra of 2-bromonaphthalene-1,4-dione in a mixture of ethanol and HEPES (5 : 5, v/v) solution were investigated upon addition of various amino acids (Cysteine, Alanine, Arginine, Aspartinie, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Proline, Serine, Threonine, Phenylalanine, Valine, Tryptophan, and Hydroxyproline). As shown in Figure 2(a), the ethanol-HEPES (5 : 5, v/v, pH of 7) solution of 2-bromonaphthalene-1,4-dione (**1**) showed two absorption bands at 348 and 452 nm. Upon the addition of CySH, the absorption band of **1** at 348 and 452 nm increased and decreased gradually, respectively. The color of the solution changed from colorless to yellow (Figure 3). In addition, the presence of two well-defined isosbestic points at 320 and 398 nm indicated the formation of thiazolidine (**2**). When other amino acids (Alanine, Arginine, Aspartinie, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Proline, Serine, Threonine, Phenylalanine, Valine, Cysteine, Tryptophan, and Hydroxyproline) were added to the solution of **1**, the spectra of 2-bromonaphthalene-1,4-dione did not induce clear spectral changes which indicated 2-bromonaphthalene-1,4-dione could be used as a colorimetric sensor for the CySH detection without the interference of other amino acids (Figure 2(b)).

3.3. Fluorescence. Fluorescence responses of 2-bromonaphthalene-1,4-dione to various amino acids were also investigated (Figure 4). As shown in Figure 4(a), the fluorescence intensity centered at 425 nm increased rapidly and shifted to 477 nm upon the addition of CySH. The fluorescence response was readily promoted because CySH was similar to 3-thiopropionic acid in chemical structure [11]. However, no obvious changes of 2-bromonaphthalene-1,4-dione were

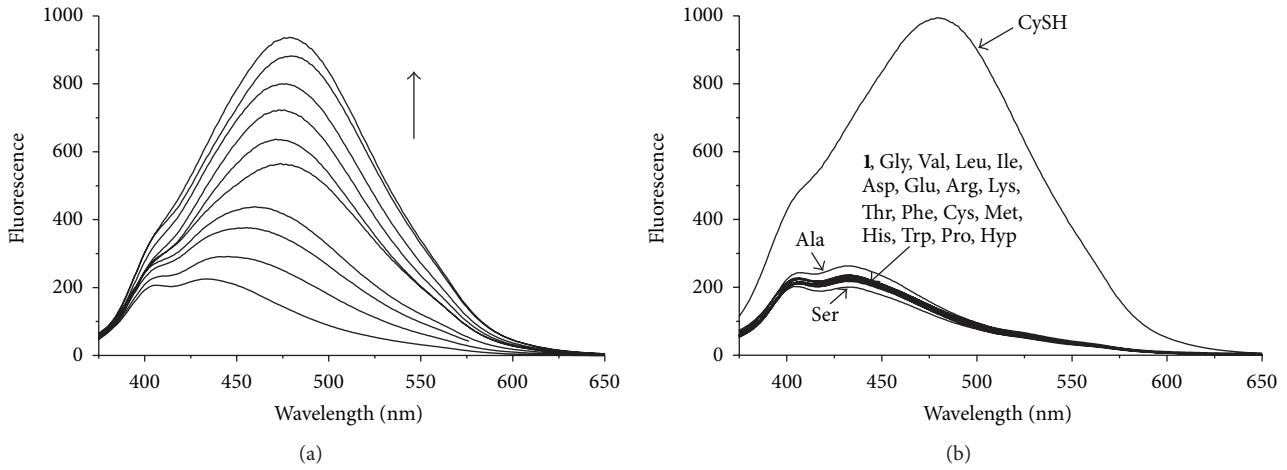


FIGURE 4: (a) Emission spectral changes of 2-bromonaphthalene-1,4-dione (2×10^{-5} mol·L^{-1}) upon addition of CySH ($0–4 \times 10^{-5}$ mol·L^{-1}) (λ_{ex} = 382 nm). (b) Emission spectral changes of 2-bromonaphthalene-1,4-dione (2×10^{-5} mol·L^{-1}) upon addition of 1 equiv. CySH, Gly, Val, Leu, Ile, Asp, Glu, Arg, Lys, Thr, Phe, Met, His, Ala, Trp, Pro, Hyp, and Ser, respectively. Condition: in ethanol-HEPES solution (5 : 5, v/v, pH of 7). The spectrum is acquired 5 min after various amino acids addition. Arrows indicate the direction of increasing CySH concentration.

observed upon the addition of other natural amino acids (Alanine, Arginine, Aspartinie, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Proline, Serine, Threonine, Phenylalanine, Valine, Cysteine, Tryptophan, and Hydroxyproline) (Figure 4(b)). These facts indicated that 2-bromonaphthalene-1,4-dione displayed a high selectivity for CySH without the interference of other amino acids.

According to Huang's study [10–12], the sensitivity of synthesized receptors was high. The further application of the above receptors was limited due to the solutions in which resolving receptors were DMF-water or methanol-water. There are three points, which showed the benefits of the previously published procedure by Huang et al. They are (i) easy preparation and low cost as 2-bromonaphthalene-1,4-dione was easily synthesized and the materials were cheap and obtained readily; (ii) water content as the water content in solution increased to 50%; however, the water content in solution was 30% or 10% in Huang's papers; and (iii) lower toxicity as the solution used in this paper was ethanol-water and the solution toxicity may be lower than other solutions such as DMF and DMSO. However, the sensor (2-bromonaphthoquinone) toward CySH detection also has disadvantage; that is, the color changes (from colorless to yellow) in the detected process were inconspicuous. This will encourage us to synthesize better sensors in order to ameliorate the deficiency of visible color changes.

3.4. Binding Constant. The sensor interacted with various amino acids as the ratio of 1 : 1 according to the Job-plot analysis. By the method of nonlinear least squares calculation, the binding constants could be obtained based on the fluorescence data [29–31]. The binding constant of the sensor with CySH was $(8.9 \pm 0.80) \times 10^5$. The binding abilities of the sensor with other amino acids tested were very weak due to the unremarkable spectral response and the corresponding binding constants can be ignored. The detection method was derived from Chen's research which was focused on the cell cytotoxicity and anticancer activity of 2-bromonaphthalene-1,4-dione derivatives. This research was focused on amino acid binding ability. As expected, the sensor (2-bromonaphthoquinone) showed high sensitivity and selectivity for CySH. In addition, the recognition process was accompanied with remarkable color changes. The above results indicated that 2-bromonaphthalene-1,4-dione could be used as a colorimetric biosensor for the CySH detection which would be a convenient method for the application of 2-bromonaphthalene-1,4-dione in pharmacy and biological samples. In the previous methods [10–12], the sensitivity of sensor with CySH was qualitative and the binding constant was not calculated. The binding constant represents the binding ability of host-guest. The bigger the binding constant was, the stronger the binding ability was. In this paper, we applied the nonlinear least squares method of calculating the binding constant of host-guest to the interaction of sensor with CySH. By calculation, the sensitivity of CySH with sensor was quantitative and also could be comparative with other sensors. The binding constant could provide a theoretical basis for the optimization of sensor and so the calculation of binding constant was necessary.

3.5. Bovine Serum Albumin Experiment. In order to examine the potential application of 2-bromonaphthalene-1,4-dione for analytical chemistry, compound **1** had been applied in the detection of Cysteine in bovine serum albumin. The UV-vis spectral changes of free CySH or bovine serum albumin were also determined which were very small and could be ignored in the tested wavelength. The UV-vis spectral response of 2-bromonaphthalene-1,4-dione in BSA was listed in Figure 5. As shown in Figure 5, the intensity of absorption peak at 350 nm was increased with the addition of BSA which showed 2-bromonaphthalene-1,4-dione interacted with Cysteine in BSA. Compared with DMSO-H_2O solution, the intensity increased slowly in BSA and the isosbestic point did not appear after the same concentration was added. However,

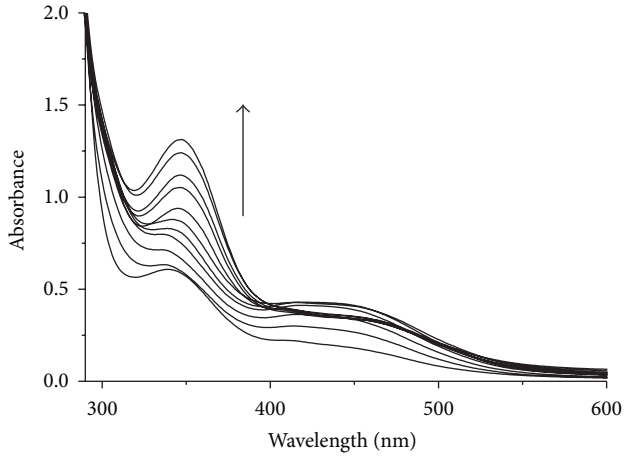


Figure 5: The UV-vis spectra changes of 2-bromonaphthalene-1,4-dione (2×10^{-5} mol·L^{-1}) with the addition of bovine serum albumin ($0–2 \times 10^{-5}$ mol·L^{-1}) at 298 K. Arrows indicate the direction of increasing CySH concentration.

the increased intensity was also remarkable. Therefore, 2-bromonaphthalene-1,4-dione can be used to detect Cysteine in BSA.

4. Conclusions

In summary, we described a highly selective chemosensor (2-bromonaphthalene-1,4-dione) for the detection of CySH without the interference of other amino acids (Gly, Val, Leu, Ile, Asp, Glu, Arg, Lys, Thr, Phe, Met, His, Ala, Typ, Pro, Hyp, and Ser) in a mixture of ethanol and HEPES (5 : 5, v/v) solution at pH of 7.0. The recognition of CySH gave obvious color changes from colorless to yellow, which was visible to the naked eye. In addition, 2-bromonaphthalene-1,4-dione also interacted with CySH in BSA. Due to the simplicity and sensitivity of the analysis, this sensor would have many opportunities in a variety of settings requiring rapid and accurate CySH detection. This understanding of the CySH sensing mechanism would actually help to find possible structural modification to achieve new probes that showed CySH sensing capacity in pure water.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81301269) and Program for Science & Technology Innovation Talents administered by the Universities of Henan Province (15HASTIT039).

References

[1] T. L. Yap, Z. Jiang, F. Heinrich et al., "Structural features of membrane-bound glucocerebrosidase and α-synuclein probed by neutron reflectometry and fluorescence spectroscopy," *Journal of Biological Chemistry*, vol. 290, no. 2, pp. 744–754, 2015.

[2] D. Polcari, A. Kwan, M. R. Van Horn et al., "Disk-shaped amperometric enzymatic biosensor for in vivo detection of D-serine," *Analytical Chemistry*, vol. 86, no. 7, pp. 3501–3507, 2014.

[3] E. Mills, E. Petersen, B. R. Kulasekara, and S. I. Miller, "A direct screen for c-di-GMP modulators reveals a *Salmonella* Typhimurium periplasmic L-arginine-sensing pathway," *Science Signaling*, vol. 8, no. 380, article ra57, 2015.

[4] S. Kumar, W. Ahlawat, R. Kumar, and N. Dilbaghi, "Graphene, carbon nanotubes, zinc oxide and gold as elite nanomaterials for fabrication of biosensors for healthcare," *Biosensors and Bioelectronics*, vol. 70, pp. 498–503, 2015.

[5] P. Batalla, A. Martín, M. Á. López, M. C. González, and A. Escarpa, "Enzyme-based microfluidic chip coupled to graphene electrodes for the detection of d-amino acid enantiomer-biomarkers," *Analytical Chemistry*, vol. 87, no. 10, pp. 5074–5078, 2015.

[6] A. Gutiérrez, I. Marzo, C. Cativiela, A. Laguna, and M. C. Gimeno, "Highly cytotoxic bioconjugated gold(I) complexes with cysteine-containing dipeptides," *Chemistry*, vol. 21, no. 31, pp. 11088–11095, 2015.

[7] Z. A. Wood, E. Schröder, J. R. Harris, and L. B. Poole, "Structure, mechanism and regulation of peroxiredoxins," *Trends in Biochemical Sciences*, vol. 28, no. 1, pp. 32–40, 2003.

[8] S. Shahrokhian, "Lead phthalocyanine as a selective carrier for preparation of a cysteine-selective electrode," *Analytical Chemistry*, vol. 73, no. 24, pp. 5972–5978, 2001.

[9] H. H. Ahmed, S. H. A. El-Aziem, and M. A. Abdel-Wahhab, "Potential role of cysteine and methionine in the protection against hormonal imbalance and mutagenicity induced by furazolidone in female rats," *Toxicology*, vol. 243, no. 1-2, pp. 31–42, 2008.

[10] D. Q. Zhang, M. Zhang, Z. Q. Liu et al., "Highly selective colorimetric sensor for cysteine and homocysteine based on azo derivatives," *Tetrahedron Letters*, vol. 47, no. 39, pp. 7093–7096, 2006.

[11] M. Zhang, M. Yu, F. Li et al., "A highly selective fluorescence turn-on sensor for cysteine/homocysteine and its application in bioimaging," *Journal of the American Chemical Society*, vol. 129, no. 34, pp. 10322–10323, 2007.

[12] M. Zhang, M. Li, Q. Zhao et al., "Novel Y-type two-photon active fluorophore: synthesis and application in fluorescent sensor for cysteine and homocysteine," *Tetrahedron Letters*, vol. 48, no. 13, pp. 2329–2333, 2007.

[13] P. R. Lima, W. J. R. Santos, C. S. Rita de et al., "An amperometric sensor based on electrochemically triggered reaction: redox-active Ar–NO/Ar–NHOH from 4-nitrophthalonitrile-modified electrode for the low voltage cysteine detection," *Journal of Electroanalytical Chemistry*, vol. 612, no. 1, pp. 87–96, 2008.

[14] M. Santhiago and I. C. Vieira, "L-cysteine determination in pharmaceutical formulations using a biosensor based on laccase from *Aspergillus oryzae*," *Sensors and Actuators B: Chemical*, vol. 128, no. 1, pp. 279–285, 2007.

[15] K. Steert, I. El-Sayed, P. Van der Veken et al., "Dipeptidyl α-fluorovinyl Michael acceptors: synthesis and activity against cysteine proteases," *Bioorganic & Medicinal Chemistry Letters*, vol. 17, no. 23, pp. 6563–6566, 2007.

[16] K. Amarnath, V. Amarnath, K. Amarnath, H. L. Valentine, and W. M. Valentine, "A specific HPLC-UV method for the determination of cysteine and related aminothiols in biological samples," *Talanta*, vol. 60, no. 6, pp. 1229–1238, 2003.

[17] H. Wang, W.-S. Wang, and H.-S. Zhang, "Spectrofluorimetic determination of cysteine based on the fluorescence inhibition of Cd(II)–8-hydroxyquinoline-5-sulphonic acid complex by cysteine," *Talanta*, vol. 53, no. 5, pp. 1015–1019, 2001.

[18] N. Burford, M. D. Eelman, D. E. Mahony, and M. Morash, "Definitive identification of cysteine and glutathione complexes of bismuth by mass spectrometry: assessing the biochemical fate of bismuth pharmaceutical agents," *Chemical Communications*, vol. 9, no. 1, pp. 146–147, 2003.

[19] G. Chwatko and E. Bald, "Determination of cysteine in human plasma by high-performance liquid chromatography and ultraviolet detection after pre-column derivatization with 2-chloro-1-methylpyridinium iodide," *Talanta*, vol. 52, no. 3, pp. 509–515, 2000.

[20] Y. V. Tcherkas and A. D. Denisenko, "Simultaneous determination of several amino acids, including homocysteine, cysteine and glutamic acid, in human plasma by isocratic reversed-phase high-performance liquid chromatography with fluorimetric detection," *Journal of Chromatography A*, vol. 913, no. 1-2, pp. 309–313, 2001.

[21] Y. Sato, T. Iwata, S. Tokutomi, and H. Kandori, "Reactive cysteine is protonated in the triplet excited state of the LOV2 domain in *Adiantum* phytochrome3," *Journal of the American Chemical Society*, vol. 127, no. 4, pp. 1088–1089, 2005.

[22] K. Kargosha, S. H. Ahmadi, M. Zeeb, and S. R. Moeinossadat, "Vapour phase fourier transform infrared spectrometric determination of L-cysteine and L-cystine," *Talanta*, vol. 74, no. 4, pp. 753–759, 2008.

[23] T. Inoue and J. R. Kirchhoff, "Electrochemical detection of thiols with a coenzyme pyrroloquinoline quinone modified electrode," *Analytical Chemistry*, vol. 72, no. 23, pp. 5755–5760, 2000.

[24] D. Potesil, J. Petrlova, V. Adam et al., "Simultaneous femtomole determination of cysteine, reduced and oxidized glutathione, and phytochelatin in maize (*Zea mays* L.) kernels using high-performance liquid chromatography with electrochemical detection," *Journal of Chromatography A*, vol. 1084, no. 1-2, pp. 134–144, 2005.

[25] S. D. Fei, J. H. Chen, S. Z. Yao, G. H. Deng, D. L. He, and Y. F. Kuang, "Simultaneous femtomole determination of cysteine, reduced and oxidized glutathione, and phytochelatin in maize (*Zea mays* L.) kernels using high-performance liquid chromatography with electrochemical detection," *Analytical Biochemistry*, vol. 339, pp. 29–35, 2005.

[26] C. Chen, Y.-Z. Liu, K.-S. Shia, and H.-Y. Tseng, "Synthesis and anticancer evaluation of vitamin K_3 analogues," *Bioorganic and Medicinal Chemistry Letters*, vol. 12, no. 19, pp. 2729–2732, 2002.

[27] S. M. McElvain and E. L. Engelhardt, "Ketene acetals. XIV. The reactions of ketene acetal with quinones," *Journal of the American Chemical Society*, vol. 66, no. 7, pp. 1077–1083, 1944.

[28] M. J. Frisch, G. W. Trucks, H. B. Schlegel et al., *Gaussian 03, Revision A.1*, Gaussian, Pittsburgh, Pa, USA, 2003.

[29] Y. Liu, B.-H. Han, and H.-Y. Zhang, "Spectroscopic studies on molecular recognition of modified cyclodextrins," *Current Organic Chemistry*, vol. 8, no. 1, pp. 35–46, 2004.

[30] Y. Liu, C. C. You, and H. Y. Zhang, *Supramolecular Chemistry*, Nankai University Publication, Tianjin, China, 2001.

[31] J. Bourson, J. Pouget, and B. Valeur, "Ion-responsive fluorescent compounds. 4. Effect of cation binding on the photophysical properties of a coumarin linked to monoaza-and diaza-crown ethers," *Journal of Physical Chemistry*, vol. 97, no. 17, pp. 4552–4557, 1993.