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Article

Application of β-Cyclodextrin/$MnFe_2O_4$ Magnetic Nanoparticles as a Catalyst for Fast Chemiluminescence Determination of Glutathione in Human Blood using Luminol-Diperiodatoargentate(III) System

Behzad Rezaei,* Ali A. Ensafi, Fariba Haghighatnia and Saeed E. Aalaye

Department of Chemistry, Isfahan University of Technology, Isfahan 84156-83111, I. R. Iran

Este trabalho baseia-se na quimioluminescência (CL) intensificada da reação entre luminol e diperiodato de prata (III) ($K[Ag(H_3IO_6)_2]$, DPA) em solução alcalina para determinação da glutadiona (GSH). Na presença de nanopartículas magnéticas do complexo de inclusão β-ciclodextrina (β-CD)/nanopartículas magnéticas $MnFe_2O_4$ (MNPs), os resultados experimentais mostraram que a emissão CL foi muito rápida (tempo de resposta de ca. 1 s, parâmetro muito importante em análise clínica) e aguda (sinal CL aumentado de 9 vezes). Os efeitos catalíticos das MNPs β-CD/MNPs $MnFe_2O_4$ na resposta CL do sistema luminol-DPA foram investigados. A linearidade do método dependeu da concentração da GSH nos intervalos de $5{,}0 \times 10^{-8}$ a $4{,}0 \times 10^{-6}$ mol L^{-1}, o limite de detecção calculado foi $1{,}5 \times 10^{-8}$ mol L^{-1} e desvio padrão relativo de 2,8% para 9 replicatas de GSH $8{,}0 \times 10^{-7}$ mol L^{-1}. O método proposto foi aplicado com sucesso na determinação rápida e sensível da GSH em amostras de sangue humano.

This work is based on the enhanced chemiluminescence (CL) of the reaction between luminol and diperiodatoargentate(III) (DPA) {$K[Ag(H_3IO_6)_2]$} in alkaline solution for determination of glutathione (GSH). In the presence of inclusion complex β-cyclodextrin (β-CD)/$MnFe_2O_4$ magnetic nanoparticles (MNPs), the experimental results showed that the CL emission was very fast (response time of about 1 s, which is a very important parameter in the clinical analysis) and sharp (CL signal increased 9 times). The catalytic effects of β-CD/$MnFe_2O_4$ MNPs on the CL response of luminol-DPA system were investigated. The linearly of method depended on GSH concentration in the ranges of 5.0×10^{-8} to 4.0×10^{-6} mol L^{-1} and limit of detection calculated was 1.5×10^{-8} mol L^{-1} and relative standard deviation of 2.8% for 9 replicated measurements of 8.0×10^{-7} mol L^{-1} GSH. The proposed method was successfully applied for rapid and sensitive determination of GSH in human blood samples.

Keywords*:* chemiluminescence, $MnFe_2O_4$ magnetic nanoparticles, glutathione, human blood, luminol-diperiodatoargentate(III)

Introduction

Aminothiols are biological agents, which are important physiologically. The levels of these compounds are important in plasma and urine as biomarkers in a number of clinical diagnoses.[1] Glutathione (GSH) is an intracellular tripeptide present in all tissues (Figure 1a). Variation in the GSH status has relationship with the pathogenesis of several diseases such as liver injury, cancer, diabetes mellitus, cataract, inflammatory diseases and radiation damages. Variation in the GSH concentration might also be due to the pollution, cigarette smoke, side effects of drugs and aging.[2] GSH can be bound to proteins or occur freely in the cells. Measurement of free GSH in the blood samples is essential to estimate the detoxification and redox status of cells because it has the protective role against oxidative and free radical-mediated radiation cell injury.[3]

A number of methods used for the determination of GSH have been reported including enzymatic,[4] spectrophotometric,[5-7] high performance liquid chromatography (HPLC),[8,9] electrochemical methods[10-18] and chemiluminescence.[19-23] The spectrophotometric methods are simple but not sufficiently sensitive. HPLC methods are usually time-consuming for sample preparation and in need of pure and sometimes toxic solvents and most of the electrochemical methods have high limits of detection.

Chemiluminescence (CL)-based detection has become in the last years quite a useful detecting tool due to the

*e-mail: rezaei@cc.iut.ac.ir

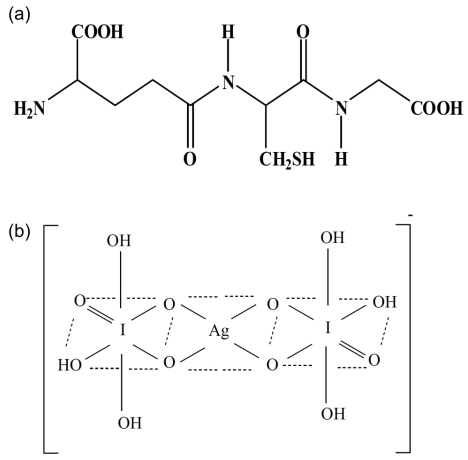


Figure 1. Molecular structures of (a) glutathione and (b) diperiodatoargentate(III) (DPA).

simplicity of detection, low limit of detection, wide linear dynamic ranges, short analysis times and relatively low cost of instrumentation which make this method appealing. One of the best and most efficient CL reactions is the oxidation of luminol in the basic solutions which can be oxidized by some strong oxidants such as diperiodatoargentate(III) (DPA). DPA, with the following complex $[Ag(HIO_6)_2]^{5-}$ in formula alkaline media, is a powerful oxidizing agent in this medium.[24]

DPA, whose chemical structure in the aqueous media is proposed in Figure 1b, has a plane-square coordinate structure around a central metal and can be easily synthesized.

Recently, it was reported a new CL system based on the reaction of $[Ag(HIO_6)_2]^{5-}$ complex with luminol for the determinations of cortisol and DPA.[25,26] The produced CL signal from luminol is followed by the formation of dinegative ions of luminol which reacts with oxygen or other oxidizing agent to yield an excited state of the aminophthalate ion responsible for the emission of light.[27] The chemiluminescent oxidation of luminol is notably enhanced, both in intensity and duration, in the presence of natural cyclodextrins (α-, β-, γ-CD). The results are explained in terms of the binding between the luminescent intermediate of the reaction, 3-aminophthalate (3-AP) and CD, rather than to the luminescent reactant itself.[28]

In recent years, more attention has been given to the metallic and magnetic nanoparticles (MNPs) because of their catalytic effect, super paramagnetic behavior, large ratio of surface to volume and low toxicity. In most investigations, magnetic nanoparticles were used for supporting catalytic action of the porous materials such as a nanostructured metal catalyst.[29-38]

The solute molecules that have the correct dimensions can interact with the cyclodextrin cavity, which is nonpolar, and form the inclusion complexes. The reduced polarity and the protection supplied by the cyclodextrin cavity have a strong and often favorable influence on the properties of the included solute.[39] On the other hand, the catalytic properties of the nanoparticles can be stabilized by β-cyclodextrin because of the interactions between metal nanoparticles and negative hydroxy groups of β-CD in water or the hydrophobic-hydrophobic interactions between metal nanoparticles and β-CD.[40] Bocanegra-Diaz *et al.*[41] showed in their studies that an inclusion compound could be formed between the magnetite and β-CD. Recently, He *et al.*[42] demonstrated the potential of β-CD as a catalyst for the CL analysis of hydrogen peroxide based on an inclusion complex of $CoFe_2O_4$ magnetic nanoparticles. In this work, the kinetic properties of the luminol-DPA system on the CL spectra of GSH were studied in the presence and absence of catalyst. It is described the novel inclusion complexes of β-CD/$MnFe_2O_4$ MNPs as catalysts for the luminol-DPA CL system. The proposed system was used for very fast, sensitive and selective CL determination of GSH in the human blood samples.

Experimental

Reagents and solution

Luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) was purchased from Merck (Germany Company). Luminol stock solution was prepared by dissolving 0.0177g of it in a specified amount of potassium hydroxide solution and then diluted with doubly distilled water to 100.0 mL to obtain the 1.0×10^{-3} mol L^{-1} concentration. β-CD (beta cyclodextrin hydrate 99%) was purchased from Acros Organics (New Jersey, USA).

DPA was synthesized according to reference considering facts such as the use of micro filter for separating precipitate and shielding the solution container from light by aluminum foil during the heating process.[24] The prepared complex, determined by the spectroscopic method, showed UV-Vis peak with maximum absorbance at 362 and 253 nm as shown in Figure 2. DPA stock solution of 2.5×10^{-4} mol L^{-1} was freshly prepared by dissolving 0.0121 g of DPA in doubly distilled water and diluting to 100.0 mL in a volumetric flask.

Stock solution of GSH (Merck, Germany) was prepared by dissolving 0.0100 g of GSH in deionized water and diluting to 10.0 mL in a volumetric flask. All of the solutions were freshly prepared and stored in a refrigerator. $MnFe_2O_4$ (MNP_S) was prepared at physics laboratory of Isfahan University of Technology (I. R. Iran).

The chemical materials used to prepare $MnFe_2O_4$ (Merck, Germany) were ferric nitrate, manganese nitrate,

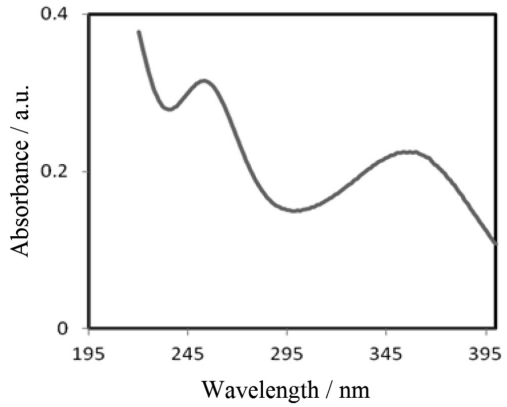


Figure 2. UV-Vis spectrum of DPA complex**.**

citric acid and ammonia. At first, an appropriate amount of metal nitrates and citric acid were dissolved in a minimum amount of deionized water to make a clear solution. Molar ratio of nitrates to citric acid was 1:1. Using this procedure, the solution was continuously stirred through a magnetic agitator. Then, the mixed solution was poured into a petri dish and heated and stirred continuously to transform into a xerogel, then the powder was heated at 300 °C for 1 h. The crystal structure of samples was analyzed using a X-ray diffractometer (Philips EXPERT MPD) with Cu-K_α (λ = 0.154 nm) radiation. The magnetic properties of the samples were determined by ac susceptibility measurements (AC susceptometer Lake Shore, Model 7000) and vibrating sample magnetometer (VSM) at room temperature. Figure 3a shows the X-ray diffraction (XRD) pattern for the sample at room temperature. The observed diffraction peaks are indexed to a cubic spinel phase. The average crystallite size of the sample was estimated by the Scherrer formula. The result showed that the average crystallite size was around 5.7 nm. Figure 3b shows the typical transmission electron micrograph of the sample. TEM image indicated that the uniform nanoparticles (about 5 nm) were well distributed and some agglomeration occurred and the ferrit nanoparticles had a tendency to agglomerate. Serum samples were taken from the Health Center of Isfahan University of Technology.

Preparation of the β-CD/$MnFe_2O_4$ MNPs

Three solutions of the inclusion complexes were prepared by mixing three samples of 0.0020 g of $MnFe_2O_4$ MNPs in the specified amount of 1.0×10^{-4} mol L^{-1} β-CD solution. Then, this β-CD solution was dissolved in three separate containers of 25.0 mL of H_2O, KOH and H_2SO_4 to yield the neutral solution, solution of 0.020 mol L^{-1} KOH and 0.010 mol L^{-1} H_2SO_4 respectively. Further, mixtures were stirred at room temperature and kept for the next use for 20 min.

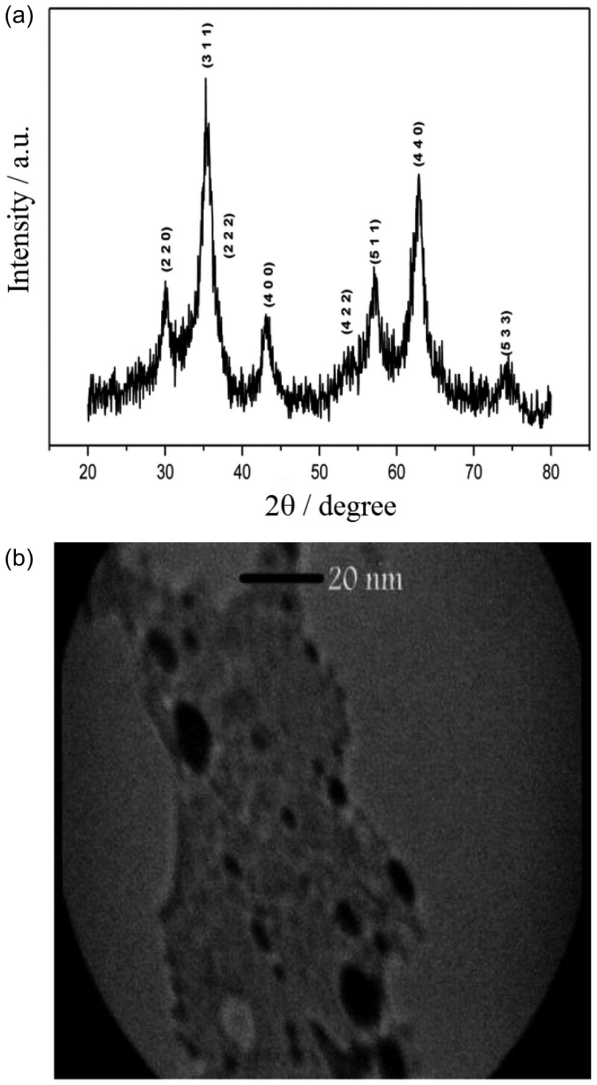


Figure 3. (a) XRD pattern of the $MnFe_2O_4$ nanoparticle sample and (b) TEM micrograph of $MnFe_2O_4$ nanoparticle.

Apparatus

The CL-batch system used in this work is shown in Figure 4.[27] It consisted of 1.0 mL quartz cell located directly in front of the window of a detector. The detector is a Hamamatsu photo multiplier tube (PMT, Tokyo, Japan Model R_{212}) connected to an amplification and filtration processing system. The CL signal was recorded in terms of time by the computer.

Spectrometric measurements were performed by UV-Vis spectrophotometer JASCO (Tokyo, Japan).

Experimental procedure

In the batch system, firstly, DPA and luminol solutions were mixed in equal volumes (250 μL) under optimum conditions to create base CL signal. When the baseline

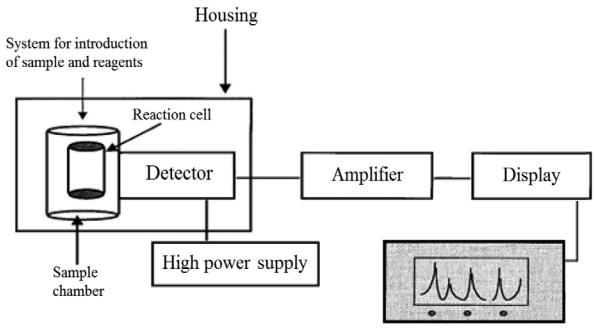


Figure 4. Schematic diagram of the CL analysis system.

became stable, GSH sample solutions (250 µL) were injected to be mixed with the reagent. The produced CL signal was detected by a photomultiplier tube (operated at −1000 V) and recorded by the computer. The CL intensity I, ($I = I_1 - I_0$, in which I_1 and I_0 were the CL intensity of sample solutions and blank solution, respectively) was converted to GSH concentration using a calibration curve.

Results and Discussion

Optimization of the chemiluminescence reaction

The CL reaction of luminol occurs in the alkaline medium. Therefore, the alkalinity of the reaction medium was regulated by preparing luminol by a suitable concentration of potassium hydroxide. The effect of potassium hydroxide concentration was examined in the range of 7.0×10^{-3} to 2.0×10^{-2} mol L^{-1} of KOH. The results were obtained using GSH concentrations in the range of 5.0×10^{-8} to 5.0×10^{-6} mol L^{-1}, DPA concentration of 2.5×10^{-4} mol L^{-1} and luminol concentration of 5.0×10^{-5} mol L^{-1} (Figure 5a). It was observed that KOH concentration of 2.0×10^{-2} mol L^{-1} had a higher CL intensity. So, this concentration was used in the magnitude studies.

The effect of luminol concentration on the CL intensity was studied in the range of 5.0×10^{-6} to 1.0×10^{-4} mol L^{-1} of luminol, KOH concentration of 2.0×10^{-2} mol L^{-1}, DPA

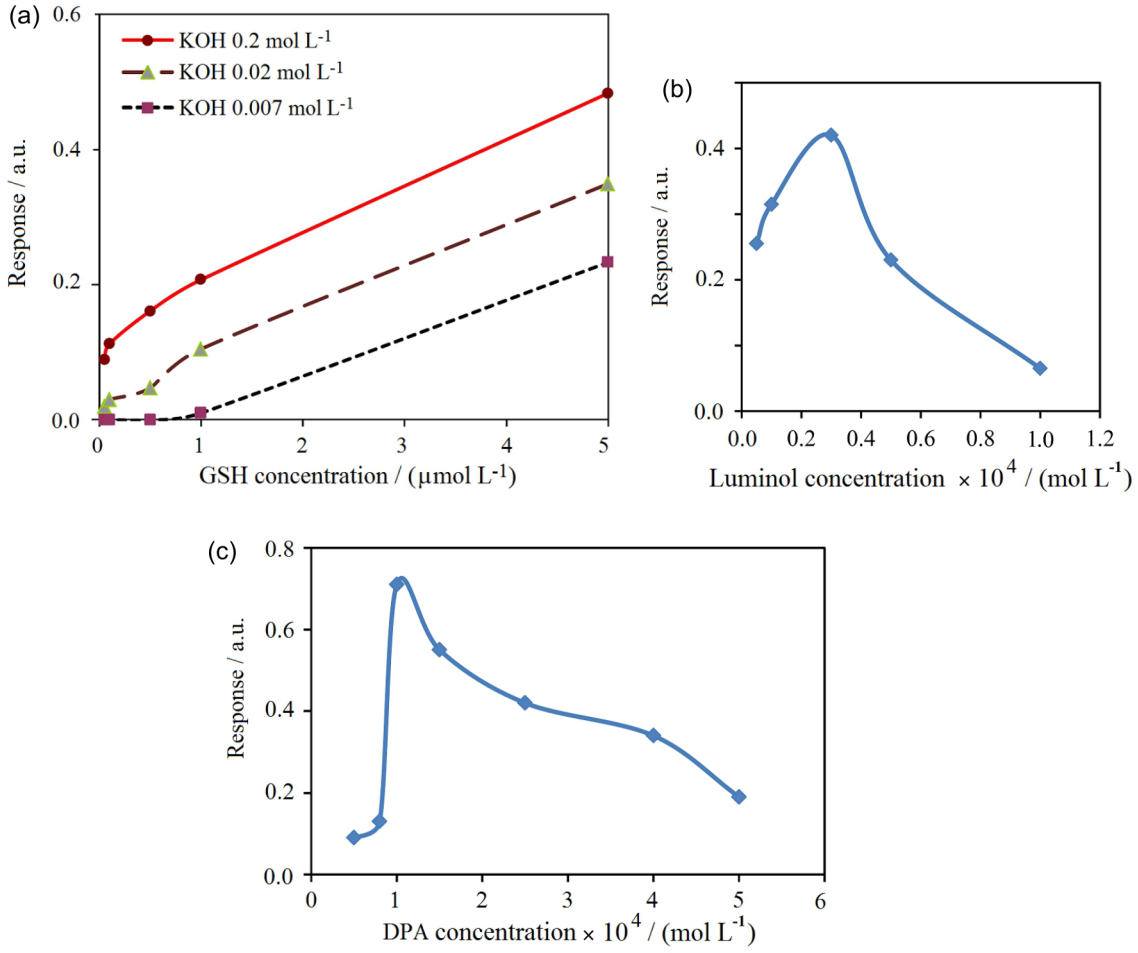


Figure 5. (a) Effect of KOH concentration on the CL intensity; conditions: [DPA] = 2.5×10^{-4} mol L^{-1}; [luminol] = 5.0×10^{-5} mol L^{-1}. (b) Effect of luminol concentration on the CL intensity; conditions: [DPA] = 2.5×10^{-4} mol L^{-1}; [GSH] = 1.0×10^{-6} mol L^{-1}; luminol in 0.020 mol L^{-1} KOH solution. (c) Effect of DPA concentration on the CL intensity; conditions: [GSH] = 1.0×10^{-6} mol L^{-1}; [luminol] = 3.0×10^{-5} mol L^{-1} (in 0.020 mol L^{-1} KOH solution).

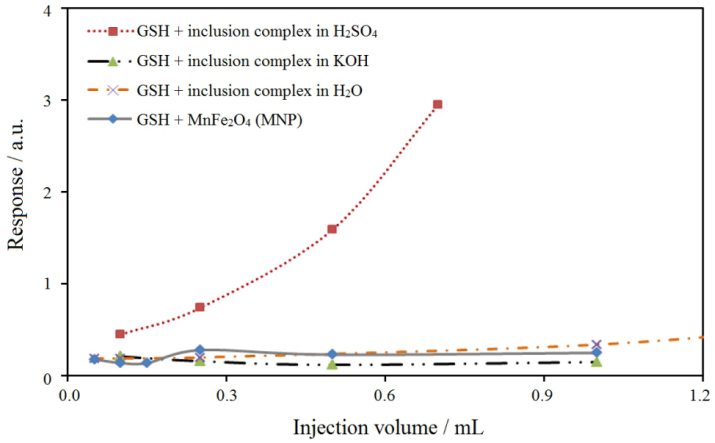


Figure 6. Effect of the amount of the inclusion complex of β-CD/$MnFe_2O_4$ MNPs and $MnFe_2O_4$ MNPs on the CL intensity in different media; conditions: [GSH] = 8.0×10^{-7} mol L^{-1}; [luminol] = 5.0×10^{-5} mol L^{-1} (in 0.02 mol L^{-1} KOH solution); [DPA] = 2.5×10^{-4} mol L^{-1}.

concentration of 2.5×10^{-4} mol L^{-1} and GSH concentration of 1.0×10^{-6} mol L^{-1} (Figure 5b). The results have demonstrated that the CL signal has increased by increasing the luminol concentration up to 3.0×10^{-5} mol L^{-1}. Additional increase in the luminol concentration decreases the CL signal. Therefore, luminol concentration of 3.0×10^{-5} mol L^{-1} was selected for further study.

The influence of DPA concentration on the CL intensity was investigated in the range of 5.0×10^{-5} to 5.0×10^{-4} mol L^{-1} using 250 μL of GSH of 1.0×10^{-6} mol L^{-1} under the optimum conditions. The results indicated that as DPA concentration increase up to 1.0×10^{-4} mol L^{-1}, CL signals increased and after that the signals decreased (Figure 5c). Therefore, DPA concentration of 1.0×10^{-4} mol L^{-1} was selected for further studies.

Catalytic influence of $MnFe_2O_4$ MNPs and β-CD on the CL signal

The variations of CL intensity for different amounts of β-CD/$MnFe_2O_4$ MNPs (controlled by different volumes of the inclusion complex injected in the specific GSH solution) were studied in the range of 0.05-1.0 mL of the inclusion complex in different media. The results showed that higher CL intensity could be achieved in the presence of β-CD/$MnFe_2O_4$ MNPs in acidic medium (Figure 6). As it was shown, an increase in the volume of the inclusion complex added to GSH solution created higher CL signals but when the volume of the inclusion complex in acidic medium was higher than 0.5 mL, the system became saturated and CL intensity was not dependent on the GSH concentration changes. Therefore, 0.5 mL of an inclusion complex in the acidic medium was selected to be added to the solutions by the specific GSH concentrations for future studies.

Analytical performance

Under the optimum conditions, dynamic ranges were obtained for the range of 5.0×10^{-8} to 4.0×10^{-6} mol L^{-1} of GSH. The CL intensity could be fitted by the equation $I = 0.9954C_{GSH} - 0.0738$ ($r^2 = 0.9885$), where GSH concentration was equal to $C_{GSH} \times 10^{-6}$ mol L^{-1} and I was the CL signal intensities (Figure. 7). The limit of detection was 1.5×10^{-8} mol L^{-1} ($3S_b/m$, S_b is standard deviation of blank and m is slope of the calibration curve) in the presence of β-CD/$MnFe_2O_4$ MNPs in the acidic medium and the relative standard deviation was 2.8% for 9 repeated measurements for GSH of 8.0×10^{-7} mol L^{-1}.

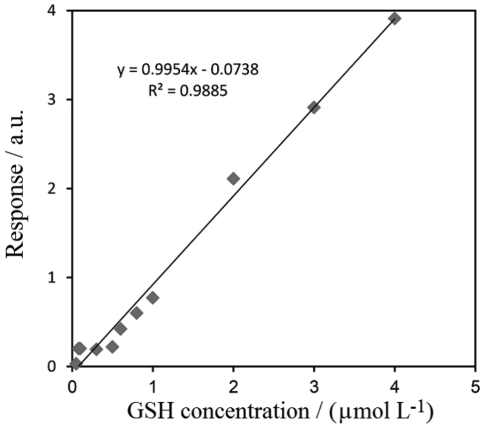


Figure 7. Calibration curve of GSH under the optimum conditions: [DPA] = 1.0×10^{-4} mol L^{-1}; [luminol] = 3.0×10^{-5} mol L^{-1} (in 0.02 mol L^{-1} KOH solution).

Interference study

In order to decrease the effects of interference substances in the analysis, the effect of diverse ions and some amino acids commonly present in the blood samples

were investigated in the determination of GSH based on the luminol-DPA CL system. The results are given in Table 1. As can be seen, the investigation started from 1000-fold concentration of each substance in proportion to GSH concentration and if there were any interference, the ratio (1000-fold) would be decreased. The tolerance of each substance was taken as the largest amount yielding an error of less than 3σ (σ is the standard deviation for 9 times determination of 8.0×10^{-7} mol L^{-1} GSH). As it is observed, ascorbic acid and methionine act as interference substances. Although these two substances are present in the plasma, the GSH is measured in erythrocyte after removing serum, thus these do not have any significant effect on the determination of GSH. It is necessary to mention that, in human blood, more than 99.5% of GSH is present in erythrocytes,[43] thus, it is possible to determine the GSH without any serious interference.

Table 1. Effect of foreign substances on the determination of GSH under optimized conditions

Substances	Substance concentration/ glutathione[a] ratio
$NaNO_3$, KCl, KNO_3, urea, aspartic acid	1000
Glucose, fructose	750
L-Cystine, NaBr, D-leucine	500
Phenylalanine, cysteine, glycine	250
Tryptophan	10
Methionine, ascorbic acid	1

[a]Glutathione concentration of 8.0×10^{-7}mol L^{-1}.

Procedure for the preparation of the blood samples

In order to determine the GSH in human blood, erythrocytes should be separated from the whole blood by removing the plasma. At first, the human blood (5.0 mL) was centrifuged for 10 min at 3000 rpm. Then, supernatant (plasma) was discarded and the rest was mixed with the 3.0 mL of NaCl 0.9% m/v. The mixture was centrifuged for 15 min at 1500 rpm and washing procedure was repeated by mixing with NaCl solutions three times in order to completely remove the plasma. Then the rest was mixed with deionized water (1:1, v/v) to hemolyzed erythrocyte pellets. Then, it was mixed with sulfosalysilic acid (10%, m/v) in the ratio of 2:1 (v/v) to make proteins precipitate. The supernatant solution was separated and centrifuged for 10 min at 3000 rpm, this was repeated three times. Then, the supernatant was collected and divided up into two parts, one part was used for the GSH determination by the spectrophotometric method and the other for GSH concentration by the chemiluminescence method. For spectrophotometric method, 500 μL of the supernatant were diluted to 10.0 mL based on the reaction of GSH and 5,5'-dithiobis-2-nitrobenzoic acid) or DTNB (Ellman's reagent).

Thiols react with DTNB to produce 2-nitro-5-thiobenzoate which is ionized to the TNB^{2-} dianion in neutral and alkaline media. In the spectrophotometric method, TNB^{2-} is quantified by measuring the absorption of light at 412 nm.[44] For the chemiluminescence method, 0.5 mL of the supernatant was diluted to 100.0 mL. Then, 5.0 mL of this solution were diluted to 50 mL until GSH concentration entered the dynamic range. Then the amount of GSH available in the solution was determined under optimum condition by the proposed CL method.

Applications

To evaluate the application of the proposed method, GSH was measured in the human blood samples. At first, the proposed CL technique was used for determination of GSH in the erythrocyte samples of humans. Also, the standard spectrophotometric method (Ellman's standard method) was used for determination of GSH in erythrocyte samples of humans. For each sample of blood, the GSH determination was repeated three times and the mean and the standard deviation of the results were recorded. The results, presented in Table 2, were compared with *t*-test and *F*-test for evaluation of the accuracy and precision of the proposed method. There is a good agreement between the results and this is a good evidence for reproducibility and accuracy of the proposed method. The obtained values were in the normal range of GSH concentration in human blood.[45]

Table 2. Concentration values obtained from the reported and reference method for glutathione analysis in human blood for three normal cases

Sample	CL method[a] ± SD / (mmol L^{-1})	Spectrophotometric method[a] ± SD / (mmol L^{-1})	t_{exp}	t_{tab} (95%)	F_{exp}	$F_{tab(2,2)}$ ($P = 0.05$)
1	1.58 ± 0.03	1.59 ± 0.02	0.57	4.30	2.25	39
2	1.11 ± 0.02	1.07 ± 0.05	3.46	4.30	6.25	39
3	1.66 ± 0.04	1.71 ± 0.03	2.16	4.30	1.77	39

[a]Mean value (n = 3). SD: standard deviation (n = 3).

Study of the chemiluminescence spectra

The determination of GSH was based on the increasing effect on the light emission generated by the oxidation of luminol by DPA in a basic medium. In order to investigate the kinetic characteristics of the reaction, CL reaction kinetics was studied. After mixing the DPA solution with luminol under the optimum condition, a baseline CL signal was created. When the baseline became stable, the GSH sample solutions were injected to that mixture and the CL reaction took place after being mixed with the reagents. The CL signal intensity reached the maximum value within about 10 s. The typical CL signals for three different concentrations of GSH were shown in Figure 8a. In order to study the role of β-CD/$MnFe_2O_4$ MNPs in the acidic medium (catalyst), 0.5 mL of this solution was mixed with an appropriate amount of GSH stock solution, which were mixed in the volumetric flask and diluted to 25 mL. Thus, different concentrations of GSH including catalyst were prepared and then injected into the solution containing the mixture of reagents in the cell and the CL signal was recorded. The CL intensity signal reached the maximum value within 1 s. Typical CL signals of 5.0×10^{-8} mol L^{-1} GSH is shown in Figure 8b with and without catalyst under the optimum conditions. As can be seen, the application of this catalyst dramatically made the CL intensity increase and response time decrease about ten times.

CL mechanism

The proposed CL mechanism for the CL reaction catalyzed by β-CD containing the inclusion complex of $MnFe_2O_4$ MNPs is shown in Scheme 1. In the first step, hydroxide ions present in the alkaline medium deprotonated DPA. In the second step, monoperiodateargentate(III) could be produced by replacement of a ligand, as shown in previous studies,[46] and then Ag^{III} oxidized GSH to produce a GSH free radical. In the previous studies, in DPA-luminol system for determination of some drugs, oxidation was occurred by DPA and a free radical was produced.[24,47] Based on the above discussion, reactions 1, 2 and 3 are proposed. It was recognized that luminol can form the anion, as shown in reaction 4 in the alkaline medium, and can be oxidized by Ag^{III} to the semidione structure (reaction 5). Based on the CL enhancement of GSH in the DPA-luminol reaction, it was recommended that GSH radical could oxidize the luminol anion to semidione structure, as shown in reaction 6, and it was a competitive reaction with reaction 5.

The semidione reacts with solute oxygen to produce the peroxy radical (reaction 8). Then, it decomposes to reach an electronically excited state by loss of nitrogen and generates

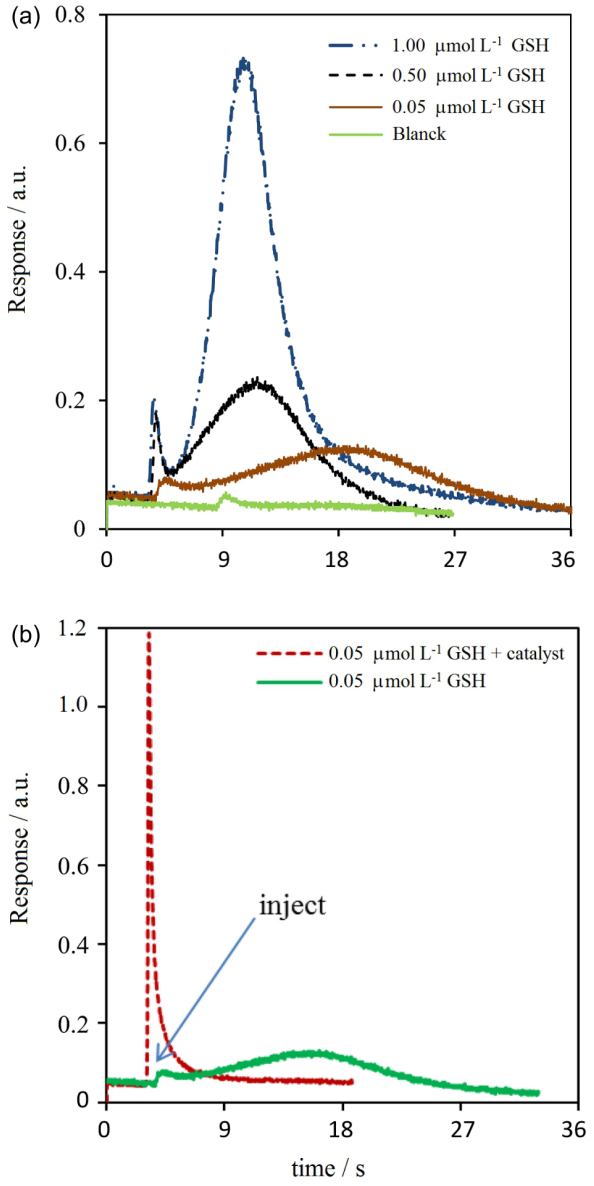
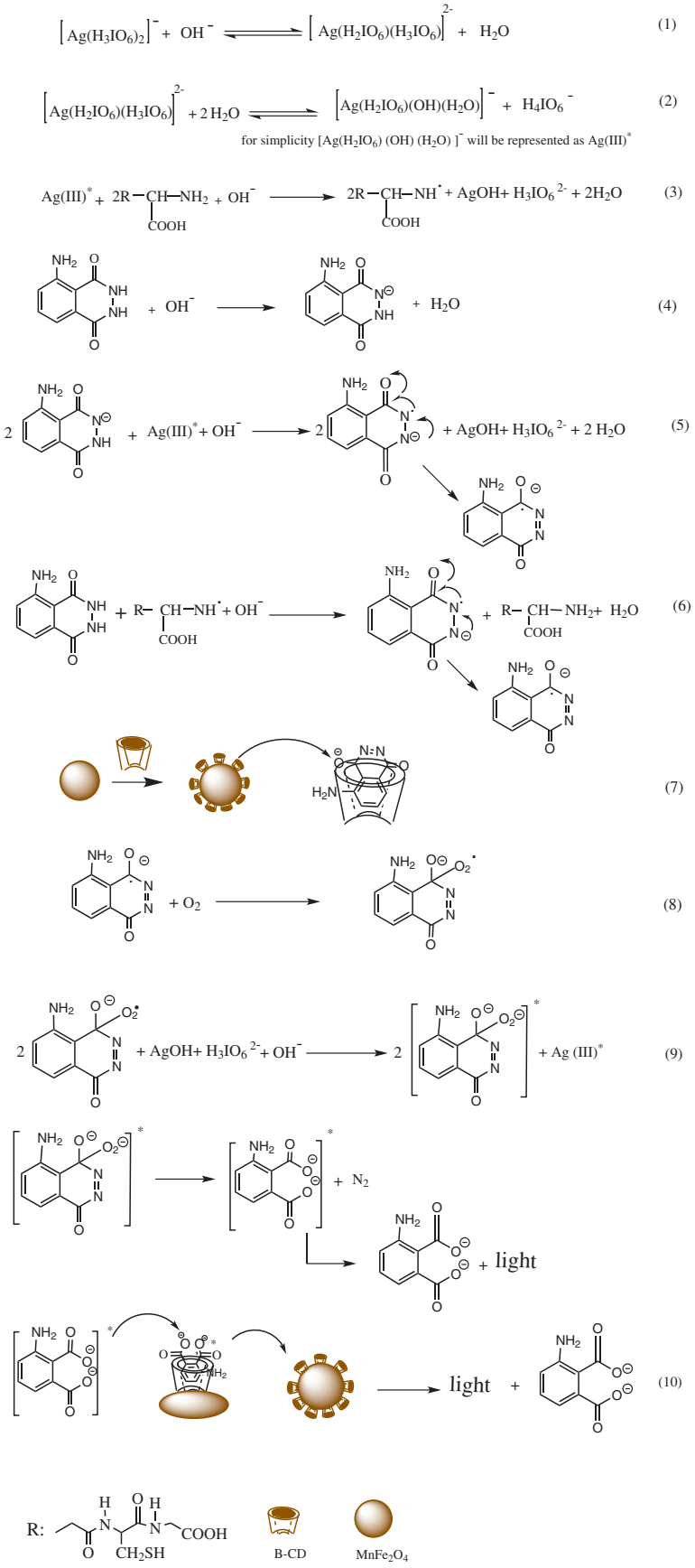


Figure. 8. (a) Typical CL signal for three different concentrations of GSH (5.0×10^{-8}, 5.0×10^{-7}, and 1.0×10^{-6} mol L^{-1}) under the optimum conditions:[DPA] = 1.0×10^{-4} mol L^{-1}; [luminol] = 3.0×10^{-5} mol L^{-1} (in 0.02 mol L^{-1} KOH). (b) Comparison signal intensity of GSH (5.0×10^{-8} mol L^{-1}) with and without the catalyst (β-CD/$MnFe_2O_4$ MNPs in 0.010 mol L^{-1} H_2SO_4) under the optimum conditions.

CL emission (reaction 9). It is possible that the basis of the CL mechanism would be without catalyst. When an inclusion complex of β-CD/$MnFe_2O_4$ MNPs in the acidic medium (catalyst) was added to GSH solution and then injected into the DPA-luminol system, CL intensity significantly increased. It can be due to the interaction between β-CD/$MnFe_2O_4$ MNPs inclusion complexes with the reactants or intermediates of the reaction of luminol with GSH. When β-CD/$MnFe_2O_4$ MNPs inclusion complex was introduced into the luminol-DPA system as the catalyst, luminol free radicals might be stabilized on the surface of β-CD/$MnFe_2O_4$



Scheme 1. Possible CL process of luminol-DPA-GSH with and without β-CD/$MnFe_2O_4$ MNPs.

Table 3. Comparison of linear dynamic ranges and limit of detections obtained by various methods

CL system	Linear dynamic range / (mol L^{-1})	Limit of detection / (mol L^{-1})	RSD / %	Reference
Lucigenin-micelle	1.0×10^{-4}-3.0×10^{-3}	2.1×10^{-5}	Not reported	20
Cu^{2+}–O_2-luminol	7.5×10^{-7}-3.0×10^{-5}	7.5×10^{-7}	1.6 (n = 5)	21
Luminol-H_2O_2	3.0×10^{-7}-2.0×10^{-5}	6.8×10^{-8}	3.4 (n = 11)	22
$Ru(phen)_3^{+2}$-$KMnO_4$	1.5×10^{-7}-1.0×10^{-5}	5.8×10^{-8}	2.2 (n = 11)	23
Luminol-DPA	5.0×10^{-8}-4.0×10^{-6}	1.5×10^{-8}	2.8 (n = 8)	this work

MNPs inclusion complex by adsorption and consequently, formation of luminol free radicals are accelerated. So, the probability of interaction of luminol free radical with oxygen which has dissolved in the solvent is increased (reaction 7). Furthermore, hydrophobic-hydrophobic interactions between the OH groups of β-CD and anion of luminol can help this process.

Moreover, the solved oxygen can be absorbed on the surface of β-CD/$MnFe_2O_4$ MNPs and makes the formation of the dianion radical molecules easy and then makes excited molecule decay very fast to emit CL photon (reaction 10). In addition, the cyclodextrin cavity can protect the excited state of the dianion of luminol from species normally occurring in an aqueous solution, which could inactivate and quench it.

When an inclusion compound of β-CD/$MnFe_2O_4$ MNPs is prepared in H_2O and/or in KOH, there would not be significant increase in the CL intensity because negative molecules of β-CD were formed in the water or in the alkaline medium repulsing the anion of luminol. Furthermore, naked $MnFe_2O_4$ MNPs could not cause significant increase in the CL intensity by itself; this indicated that, in the present CL reaction, $MnFe_2O_4$ MNPs had no catalytic effects by itself.

Response characteristic

In the proposed method, β-CD/$MnFe_2O_4$ MNPs is recommended as a novel catalyst which has an enhanced role in the CL intensity of luminol-DPA reaction and also decreases the speed of GSH analysis time more than ten times. The results of the proposed method for GSH determination is compared with the other CL methods presented in Table 3. As it is shown, the proposed method had a better dynamic range and lower limit of detection and, most importantly, its response time was shorter in comparison with the proposed methods.

Conclusion

In this study, a novel class of inclusion compounds is presented. They are inclusion complexes of β-CD/$MnFe_2O_4$ MNPs in the acidic medium as catalysts for the CL system using luminol-DPA as a model. The use of this catalyst increases dramatically (9 times) the CL intensity and the response time (the time that peak has reached its maximum level) decreases about ten times. The current investigation has demonstrated the potential of inclusion complexes based on β-CD/$MnFe_2O_4$ in the acidic medium as an efficient enhancement reagent for the luminol CL system.

Measurement of GSH level in human blood is a benefit as it serves as a biomarker for some diseases. The present method is capable of measuring GSH in blood and is statistically precise in comparison with the Ellman's reference method. These facts suggest the luminol-DPA CL reaction can be a promising detection system for HPLC or capillary electrophoresis in GSH determinations in biological medium. The proposed method has prominent advantages including instrumental simplicity, high sensitivity and selectivity and an easy handling procedure.

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