J Fluoresc (2013) 23:227–235
DOI 10.1007/s10895-012-1138-x

ORIGINAL PAPER

Determination of Epinephrine in Pharmaceutical Formulation by an Optimized Novel Luminescence Method Using CdS Quantum Dots as Sensitizer

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Received: 8 July 2012 / Accepted: 2 October 2012 / Published online: 11 October 2012

Abstract In this article, a novel chemiluminescence method using water-soluble CdS quantum dots (QDs) as sensitizers is proposed for the chemiluminometric determination of epinephrine. The method is based on the quenching effect of epinephrine on the chemiluminescence emission generated by the mixing of CdS quantum dots (QDs) with hydrogen carbonate (HCO_3^-) in the presence of hydrogen peroxide (H_2O_2) in an alkaline medium. The optimization of variables influencing the chemiluminescence response of the method has been carried out by using experimental design. Under the optimal conditions, there is good linear relationship between the relative chemiluminescence intensity and the concentration of epinephrine over the range of 5×10^{-9}–1×10^{-6} molL^{-1} with a 3σ detection limit of 5×10^{-11} molL^{-1}. The method has been successfully applied to the determination of epinephrine in pharmaceutical formulation and the recovery test was done in human urine.

Keywords CdS quantum dots · Epinephrine · Chemiluminescence · Quenching · Experimental design

Introduction

Epinephrine (EP), often called adrenaline, is an important catecholamine neurotransmitter in mammalian central nervous systems, and it exits in the nervous tissue and body fluid in the form of large organic cations [1]. Medically, EP has been used as a common emergency healthcare medicine [2]. Many phenomena are related to the concentration of epinephrine in blood as well as urine. Also, low levels of EP have been found in patients with Parkinson's disease [3]. There are some methods applied for the determination of EP, such as high performance liquid chromatography (HPLC) [4], capillary electrophoresis [5], electrochemiluminescence [6], chemiluminescence [7], fluorimetry [8, 9] and electrochemistry [10, 11]. As the quantitative determination of EP concentration is significant for developing nerve physiology, pharmacological research and life science [12]; therefore, it is very much essential to develop simple and suitable analytical methods for its estimation in bulk and in formulations. In recent years semiconductor nanocrystals, known as quantum dots (QDs), are in high-demand as inorganic fluorophores [13]. Several advantages, including flexible photoexcitation, sharp photoemission, and excellent resistance to photobleaching have made them more attractive than conventional organic fluorophores as luminescent molecular probes [14, 15]. As the optical properties of QDs strongly depend on the nature of their surface, modifications of the surface with functional groups or biomolecules and the interactions that it could establish with specific analytes can result in dramatic changes in these properties [16]. Thus, fluorescence or chemiluminescence (CL) based chemical sensing involving QDs have been developed for different chemical species such as ascorbic acid [17], urea [18], sulfadiazine [19], as well as aions, such as fluoride, chloride and acetate ions [20]. In most QDs applications, the detection is based on signal quenching, while more newly attention has been focused

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on signal enhancing, mainly related to QD ability to sensitize different chemiluminescent systems [17, 21, 22]. Sensitized chemiluminescence is an expeditious policy to exploit CL reactions with low quantum efficiencies for analytical purposes. The weak created energy is transferred to a sensitizer, usually an organic fluorophore with high quantum yield, which is able to magnify it. Any species that selectively interacts with the fluorophore could quench the CL emission. To our knowledge, up to know, there is no report on sensitize effect of CdS QDs on distinct chemiluminescent systems. During CL analysis, a large number of factors might affect the result. This indicates the necessity to appropriately optimize these factors and obtain measurements under the best possible conditions. Similar to all analytical methods, the factors involving CL measurements are not commonly independent. Thus, if "one-at-a-time" optimization (i.e., univariate optimization) is used, a biased interpretation of the system being studied might be obtained. The use of multivariate experimental design techniques is becoming more and more widespread in analytical chemistry. Multivariate experimental design techniques, which allow the simultaneous optimization of several variables, are faster to implement and more cost-effective than traditional univariate (one at a time) approaches [23]. However, the use of experimental design in the optimization of influence factors on chemiluminescence of QDs has not been reported. In the present study, we have found that the oxidation of HCO_3^- by H_2O_2 in alkali media and in the presence of CdS QDs that act as sensitizers produces strong CL signal to allow the development of detection systems. This paper presents a rapid, simple and sensitive method for measuring epinephrine in pharmaceutical formulation and the recovery test was done in human urine that could be more convenient for clinical use.

Experimental Section

Chemicals

All the reagents or solvents were of analytical grade and used without further purification. Ultrapure water (deionized and doubly distilled) was used throughout. Cadmium chloride hydrate, sodium hydroxide and hydrogen peroxide (H_2O_2, %) were purchased from Merck (Darmstadt, Germany). Thioglycolic acid (TGA), sodium hydrogen carbonate ($NaHCO_3$) and sodium carbonate (Na_2CO_3) were from Fluka (Buchs, Switzerland). Sodium sulfide was from Acros (Geel, Belgium). Epinephrine was purchased from Sigma-Aldrich (ST. Gallen, Germany). Stock standard solution of epinephrine (1×10^{-3} molL^{-1}) was prepared by dissolving 0.0183 g epinephrine in 100 ml 0.05 molL^{-1} sodium hydroxide and stored in dark bottles at 4 °C in a refrigerator. Working standard solutions were prepared daily by diluting the stock solutions with distilled water just before use. Epinephrine hydrochloride injection labeled as containing 1.0 mgmL^{-1} of EP is available and was used. A buffer solution of pH 10.55 was prepared by mixing Na_2CO_3 (0.5 molL^{-1}) and $NaHCO_3$ (0.5 molL^{-1}) solutions in a volume ratio of 9:1. Fresh working solutions of H_2O_2 were prepared daily from 30 % (v/v) H_2O_2 and were standardized by titration with a standard solution of $KMnO_4$.

Apparatus and Methods

UV–Vis absorbance spectra of CdS nanocrystals were obtained from aqueous CdS QDs solutions using a Cecil CE5501 spectrophotometer (Cambridge, UK). Photoluminescence (PL) measurements were recorded on a Perkin Elmer LS- 3B Luminescence Spectrometer (Waltham, USA) using 10 mm quartz cuvettes. Excitation wavelength was set at 390 nm. The CL light intensity time curve was obtained on Bertholddetection systems, Sirius-tube luminometer (Pforzheim, Germany). All optical measurements were carried out at room temperature. Transmission electron microscopy (TEM) images of the as-prepared QDs were obtained by using a Philips CM10 system (Andover, USA).

Synthesis of TGA-Capped CdS Quantum Dots

Thioglycolic acid (TGA)-stabilized CdS QDs were synthesized via arrested precipitation in water as described previously [24]. Nano crystals were prepared from a stirred solution of $CdCl_2$ (5 mM) in 100 mL of pure water. The pH was lowered to 2.15–2.30 with thioglycolic acid and then raised by dropwise addition of concentrated 10 M NaOH to pH 4.5, followed by further dropwise addition of 1 M NaOH to obtain a final desired pH of 7.0±0.05. The solution was stirred vigorously under nitrogen atmosphere for 30 min. Then, 20 mL of 12 mM $Na_2S.9H_2O$ aqueous solution was added to this solution with rapid stirring, in order to set the molar ratio of Cd^{2+}/S^{2-} to 1:0.4. The reaction mixture was stirred for 4 h prior to analysis. Particles with various sizes were obtained by varying either the pH before adding the $Na_2S{\cdot}9H_2O$ solution or the $[CdCl_2]$:$[Na_2S]$ molar ratio. The final concentration of the CdS QDs was approximately 4×10^{-3} molL^{-1} (according to the Cd^{2+} concentration). For purification of CdS QDs, the colloid solution was dialyzed against 0.01 M NaOH solution for 2 days. A dialysis membrane with a molecular weight of cutoff 7000 was used for the purification of CdS QDs.

Analytical Procedure

Solution A was made by mixing 200 μL of CdS QDs (appropriate concentrations in water), 200 μL of buffer (appropriate concentrations in water) and 100 μL water or 100 μL epinephrine (various concentrations in water). Solution A was delivered to the instrument quartz cuvette via polypropene syringes. Then 50 μL proper concentration of hydrogen peroxide solution in water was injected into the quartz cuvette and the chemiluminescence spectrum was recorded.

Experimental Design

Full Factorial Design

A two-level full factorial design contains all possible combinations between the factors and levels. These designs allow estimating all main (i.e. of the factors) and interaction effects between the considered factors [23]. The full factorial design was based on the first-order model:

$$Y = \beta_0 + \sum \beta_i x_i \tag{1}$$

Where Y is the response, β_0 is the model intercept and β_i is the linear coefficient, and x_i is the level of the independent variable [25]. The purpose of the full factorial design was to select the significant factors affecting the response in the analytical procedure. In this study, a four factor, two-level full factorial design was generated with the assistance of Statgraphics 5.1 software and performed with the four factors being H_2O_2 concentration, QDs concentration, buffer concentration and pH. For the design setup, two different coded levels for each factor were used and the corresponding uncoded values are shown in Table 1. The levels of these factors were chosen based on preliminary experiments.

Box–Behnken Design

The optimum conditions for maximizing the CL emission intensity were determined by means of a Box–Behnken experimental design combining with response surface modeling (RSM) and quadratic programming. Box–Behnken designs (BBD) are a class of rotatable or nearly rotatable second-order designs based on three-level, incomplete factorial designs [26]. The number of experiments (N) required for BBD development is defined as:

$$N = 2K(K-1) + C_0 \tag{2}$$

Where K is the number of factors and C_0 is the number of central points [27]. In this study, the most important factors that found from screening design are H_2O_2 concentration, QDs concentration and pH. The maximum and minimum levels of the factors were the same as in the screening step. These three significant factors are used to determine the optimal conditions and examined in more detail using response surface designs. The Box–Behnken design has 15 experimental runs with three runs at the center point (Table 2). The experimental data was analyzed with Statgraphics 5.1 software and fitted into a second-order equation. The quadratic equation model is as the following:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j + \varepsilon \tag{3}$$

where Y is the process response or output (dependent variable), k is the number of the patterns, i and j are the index numbers for pattern, β_0 is the free or offset term called intercept term, x_1, x_2,. . ., x_k are the coded

Table 1 Factors in actual and coded levels for the full factorial design and optimization (BBD)

Variables	Symbol	Low(−)	Middle (0)	High(+)
H_2O_2 concentration (M)	A	0.05	0.52	1
CdS QDs concentration (M)	B	10^{-6}	5×10^{-5}	10^{-3}
Buffer concentration (M)	C	0.1	0.45	0.8
pH	D	8	9.5	11

Table 2 Design matrix and corresponding response variable (chemiluminescence) for the Box–Behnken design

No.	Parameters			Mean chemiluminescence $\times10^4$
	H_2O_2 concentration (A)	CdS QDs concentration (B)	pH (D)	
1	0	−1	1	350
2	0	0	0	348
3	1	0	−1	226
4	0	1	1	294
5	−1	0	1	349
6	0	0	0	342
7	−1	−1	0	328
8	0	0	0	352
9	1	−1	0	315
10	1	1	0	247
11	−1	1	0	283
12	0	−1	−1	250
13	−1	0	−1	252
14	0	1	−1	211
15	1	0	1	342

independent variables, β_i is the first-order (linear) main effect, β_{ii} is the quadratic (squared) effect, β_{ij} is the interaction effect, and ε is the random error or allows for discrepancies or uncertainties between predicted and measured values [28].

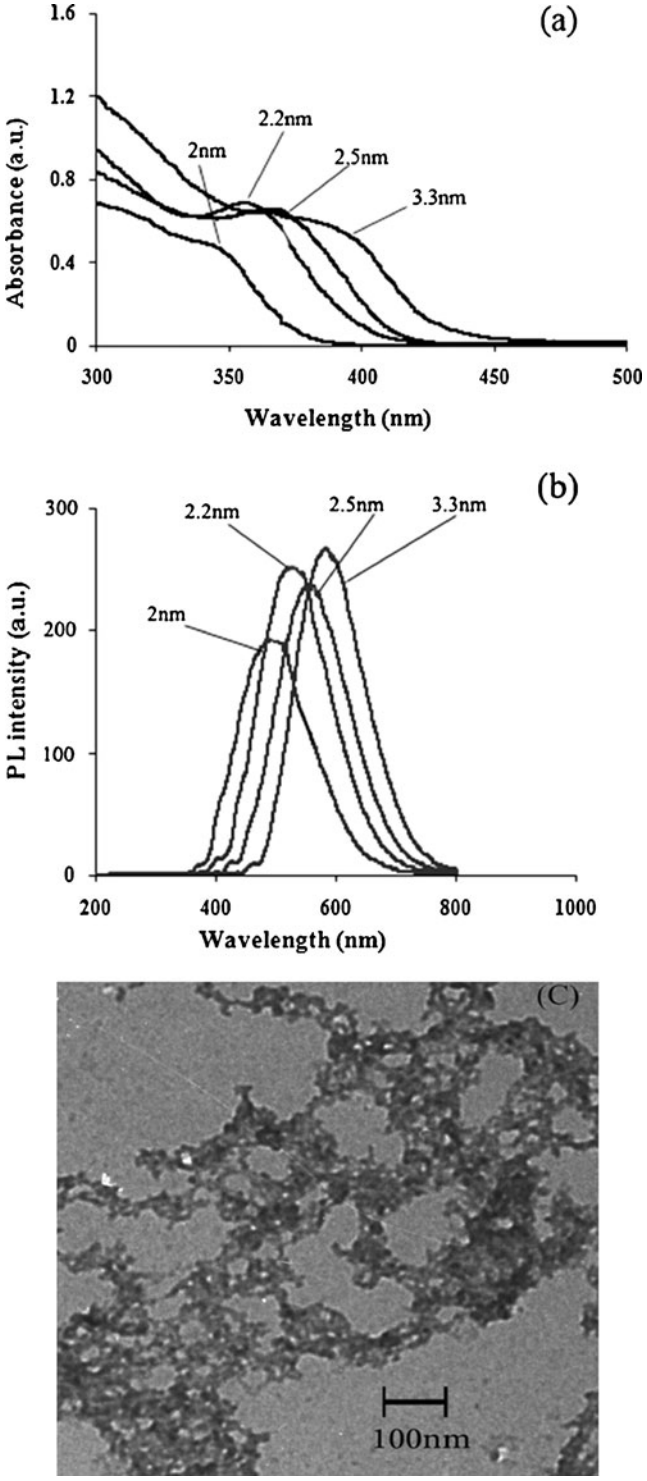

Fig. 1 Characterization of CdS QDs. **a** UV–Vis absorption spectra; **b** photoluminescence spectra (the excitation wavelength is 390 nm); **c** TEM image of CdS QDs

Results and Discussion

Absorption and Photoluminescence Spectra of CdS QDs

The UV–vis and PL spectra are powerful tools to confirm quantum-confined property of semiconductor QDs. Figure 1 illustrates the absorbance and room temperature PL spectra of CdS nano crystals of different sizes. All as-prepared colloids showed a well-resolved absorption maximum of the first electronic transition, indicating narrow size distribution of the CdS QDs. The photoluminescence peak and absorption maximum shifted to longer wavelength with increasing Nano crystals (NCs) sizes as a result of quantum confinement effects. Diameter of CdS QDs was calculated as [29]:

$$D = \left(-6.6521 \times 10^{-8}\right)\lambda^3 + \left(1.9557 \times 10^{-4}\right)\lambda^2 - \left(9.2352 \times 10^{-2}\right)\lambda + 13.29 \quad (4)$$

Where D (nm) is the diameter of the Nano crystals and λ (nm) is the wavelength corresponding to maximal absorbance. The size of the quantum dots are around 2, 2.2, 2.5 and 3.3 nm, respectively, corresponding to the first excitonic absorption peaks of 348, 360, 370 and 398 nm. Figure 1c shows the typical TEM image of CdS QDs.

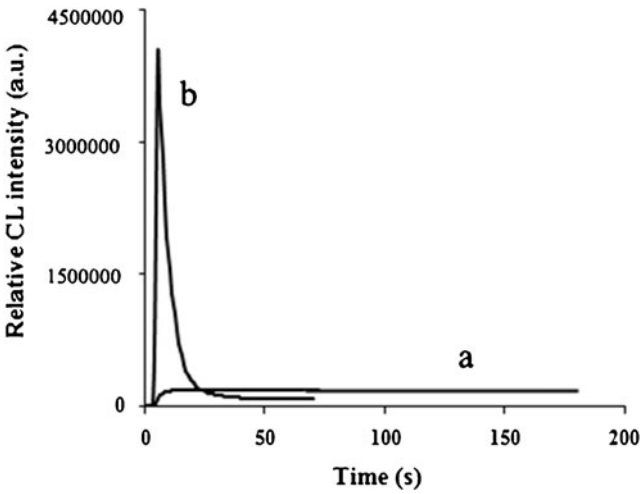

Fig. 2 Dynamic CL intensity–time profiles of $NaHCO_3$-H_2O_2 (**a**), $NaHCO_3$-H_2O_2-CdS NCs (**b**). Conditions: **a** 50 μL H_2O_2 solution (proper concentration) was injected into a 500 μL buffer solution (pH=10.55); **b** 50 μL H_2O_2 solution (proper concentration) was injected into a mixture of 200 μL CdS QDs (proper concentration) with a particle size of around 3.3 nm and 200 μL buffer solution (pH=10.55) plus 100 μL water

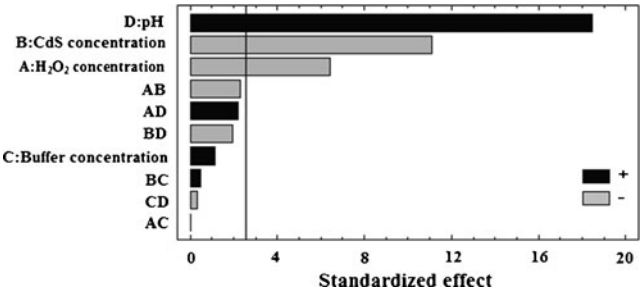

Fig. 3 Pareto chart of the main effects obtained from 2^4 factorial design

Chemiluminescence of CdS QDs

Chemiluminescence emission of CdS NCs was studied in $NaHCO_3$-H_2O_2-CdS QDs system. It was reported that peroxymonocarbonate ion (HCO_4^-) is a luminous species and can be generated in HCO_3^-–H_2O_2 system [30, 31]. However, HCO_4^- provided a weak chemiluminescence emission, which can be enhanced in the presence of sensitizers or fluorophore compounds [32]. Several compounds can be used, and special attention has been given to QDs due to their high quantum yields [17]. Therefore, in this study we investigate the effects of CdS NCs on the $NaHCO_3$-H_2O_2 CL system. Figure 2 shows the dynamic CL intensity-time profiles of the $NaHCO_3$-H_2O_2 (curve a) and $NaHCO_3$-H_2O_2-CdS NCs (curve b) were acquired in static-injection mode. It indicated (Fig. 2) that the CL reactions were very quick and the CL intensity reached a maximum in about a second after the injection. It could be seen from Fig. 2 that the CL intensity of $NaHCO_3$-H_2O_2-CdS NCs system is far stronger than that of $NaHCO_3$-H_2O_2 system, indicating the great sensitized effect of CdS NCs on $NaHCO_3$-H_2O_2 CL reaction. Parameters influencing the CL signals of $NaHCO_3$-H_2O_2-CdSNCs system were then investigated systematically to establish the optimal conditions for the CL reaction. As many factors mixed up in the optimization of this analytical method, experimental design was used to create the developing process more efficient and cost-effective. This design was carried out in the following experiment.

Table 3 Analysis of variance table (ANOVA) of BBD design

Source	Sum of squares	d.f.[a]	Mean squares	*F*-value[b]	*p*-value[c] prob > F
A	840.5	1	840.5	17.83	0.0083
B	5,408	1	5,408	114.74	0.0001
D	19,602	1	19,602	415.88	0.0000
AA	1338.78	1	1338.78	28.40	0.0031
AB	132.25	1	132.25	2.81	0.1548
AD	90.25	1	90.25	1.91	0.2250
BB	4533.85	1	4533.85	96.19	0.0002
BD	72.25	1	72.25	1.53	0.2706
DD	4796.31	1	4796.31	101.76	0.0002
Residual	235.6667	5	47.1333		
Pure error	185	2	25.3333		
LOF[d]	50.6667	3	61.6667	2.43	0.3045

[a] Degrees of freedom

[b] Test for comparing model variance with residual(error) variance

[c] Probability of seeing the observed *F*-value if the null hypothesis is true

[d] The variation of the data around the fitted model

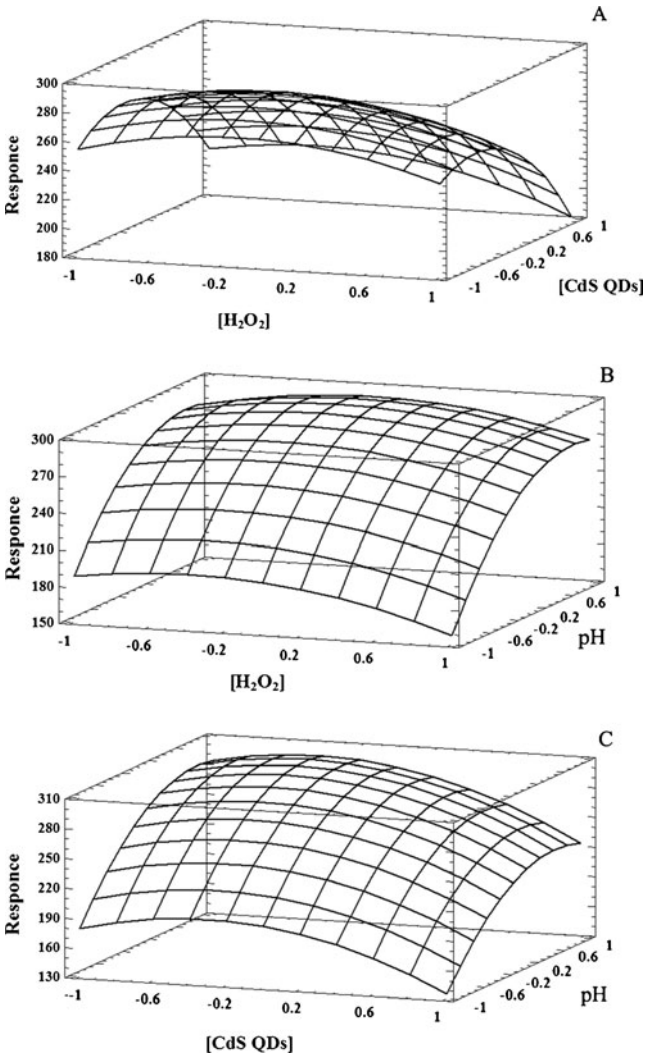

Fig. 4 **a** Response surface of the effects of H_2O_2 concentration and CdS QDs concentration on the response (CL emission intensity of $NaHCO_3$-H_2O_2-CdS QDs system), with the pH fixed at the coded level 0 (actual level 9.5). **b** Response surface of the effects of H_2O_2 concentration and pH on the response, with the CdS QDs concentration at the coded level 0 (actual level 5×10^{-5} M). **c** Response surface of the effects of CdS QDs concentration and pH on the response, with the H_2O_2 concentration fixed at the coded level 0 (actual level 0.52 M)

Optimization

Full Factorial Design

Determination of significant factors affecting the response is the first step in optimization of the experimental conditions. In order to perform a preliminary analysis, a two-level full factorial design was used. Four factors were evaluated, including the H_2O_2 concentration, QDs concentration, buffer concentration and pH. The CL emission intensity of QDs (peak height) considered as the experimental response. The high and low levels of these factors were chosen based on previous experiments (Table 1). To simplify the experiments, size effect consider as independent parameter and was kept constant because the results revealed that the particle size have no effect on the kinetics of the CL reaction; increasing these size only enhances the CL intensity. The highest CL intensity was obtained by the CdS QDs with 3.3 nm in diameter so this particle size was chosen for further experiments. Herein, we reported on an application of a full factorial design (2^4) with 16 experiments to explore important factors and their interactions. The experiments were run randomly to minimize the effect of uncontrolled variables. Treatment of the obtained data using analysis of variance (ANOVA) and statistical probability (p=0.05) resulted in the Pareto chart shown in Fig. 3. The bar length in the chart is proportional to the absolute value of the standardized effect of that factor on the response. The results (Fig. 3) demonstrated that H_2O_2 concentration (A), QDs concentration (B) and pH (D) were the most significant variables and were evaluated in the BBD for further assessment. The negative coefficients of A and B show that they have negative effects on the CL emission intensity. In addition, the P values of C (buffer concentration) demonstrated that the buffer concentration had no significant effect on the CL emission intensity of QDs and was eliminated for further studies (BBD). As it is represented in Fig. 3 the buffer concentration has positive sign; therefore, due to the limited solubility of $NaHCO_3$ in water, its concentration was kept constant at medium level (0.5 $molL^{-1}$), which gave reasonable peak heights after injection.

Box–Behnken Design Experiment

Based on the results of factorial design experiments, the optimization was performed using response surface methodology with Box–Behnken design. Three variables were examined and simultaneously evaluated, concerning the H_2O_2 concentration (A), QDs concentration (B) and pH (D). The maximum and minimum levels of the factors were the same as in the screening step. The central point was taken as the arithmetic mean between minimum and maximum level values for each variable. A total of 15 experiments were required for the optimization process. The design matrix and the responses are illustrated in Table 2. The experiments were run in random manner to overcome the effects of uncontrolled factors. The Statgraphics 5.1 software was used to analyze the experimental results. The analysis of variance (ANOVA) was done to test the significance of the model as given in Table 3.

As well-known in Table 3, the ANOVA of the regression model, explained that the P-value of the model was significant (P<0.05), while the lack of the fitted value of the model was 0.3045 (P>0.05, not significant). Both of the values indicated that the regression model was valid for the present study. As can be seen in this table, the most significant variables are: concentration of H_2O_2, concentration of QDs and pH. By fitting multiple regression analysis on the design matrix and the responses given in Table 2, the following

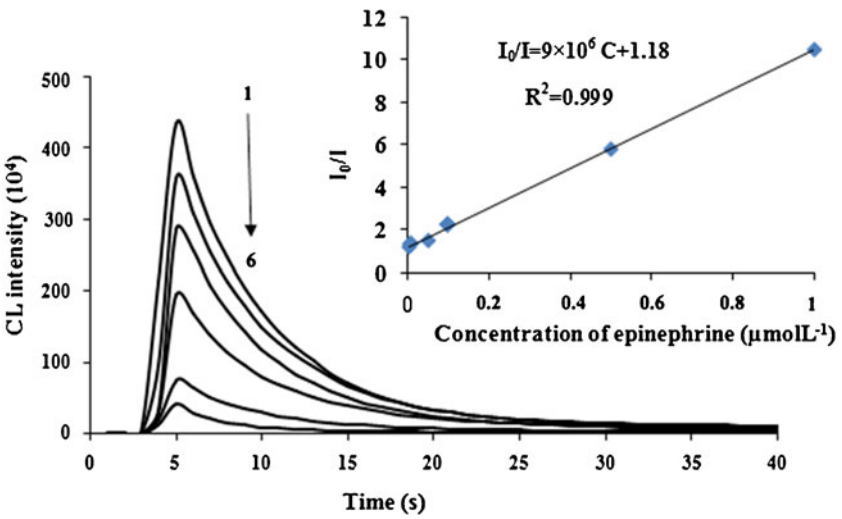

Fig. 5 The changes of the CL spectra of $NaHCO_3$-H_2O_2-CdS QDs system after addition of various concentrations of epinephrine. The solution conditions were: 0.46 $molL^{-1}$ H_2O_2, pH=10.55 and 2.9×10^{-4} $molL^{-1}$ CdS QDs with different concentrations of epinephrine: (1) 0.0, (2) 0.005, (3) 0.01, (4) 0.1, (5) 0.5, (6) 1 $\mu molL^{-1}$. The inset shows linear dependence of relative chemiluminescence intensity (I_0/I) as a function of epinephrine concentration ($\mu molL^{-1}$)

second-order polynomial equation in coded form was created:

$$Y = 277.333(\pm 3.84) - 12.875(\pm 2.63)A - 26(\pm 2.35)B + 46.875(\pm 2.63)D - 21.667(\pm 3.65)A^2 - 32.417(\pm 3.65)B^2 - 38.667(\pm 3.65)D^2 \quad (5)$$

$R^2 = 0.991$, SE = 6.65, n = 15

Where Y is the CL emission intensity of $NaHCO_3$-H_2O_2-CdS QDs system, A, B and D are the symbols for H_2O_2 concentration, QDs concentration and pH respectively. The coefficient of determination (R^2) value was 0.991. This result revealed that model could explain 99.1 % of the variability in the response. By solving the Eq. 5 and also by analyzing three-dimensional response surface (Fig. 4), the following optimum values of these significant factors were obtained: a 0.46 M H_2O_2 concentration, a 2.9×10^{-4}M CdS QDs concentration and a 10.55 pH value. In this condition the highest CL emission peak heights was achieved.

Effect of Variables on CL Intensity (Three Dimensional (3D) Response Surfaces)

In this study, in order to achieve a better understanding of the effects of the independent variables and their interactions on CL emission intensity of CdS QDs, 3D response surface plots for the measured responses were generated based on the model equation (Eq. 5). As the regression model has three independent variables, one variable was kept at constant at the center level for each plot, thus, a total three response 3D plots were formed for responses. Figure 4 shows the 3D response surfaces as the functions of two variables at the center level of other variables. The nonlinear character of all 3D response surfaces confirmed that there were considerable interactions between each of the independent variables and the CL emission intensity.

Table 4 Comparison of the linear ranges and detection limits for epinephrine assay in pharmaceutical formulation by the proposed method and other reported methods

Methods	DL (molL^{-1})	LDR (molL^{-1})	Ref.
Electrochemiluminescence (ECL)	2.4×10^{-8}	4.0×10^{-8}–2.0×10^{-7}	[6]
Fluorimetriy	2.4×10^{-10}	1.4×10^{-9}–2.1×10^{-6}	[8]
Electrochemistry	4.4×10^{-7}	0.9×10^{-6}–2.16×10^{-4}	[11]
This work	5×10^{-11}	5×10^{-9}–1×10^{-6}	

Table 5 The determination of epinephrine in the injection

Formulation	Claimed value (mg/ml)	Found	Recovery (%)	RSD (n=3, %)
Epinephrine injection (1 mg/ml)	1	0.988	98.8	3

The CL Reaction

The reaction of hydrogen carbonate (HCO_3^-) with hydrogen peroxide (H_2O_2) in basic medium yields a weak chemiluminescent emission, which can be enhanced in the presence of sensitizers or fluorophore compounds, one of which is QDs that attract special attention due to their high quantum yields [30–32]. Chen et al. proposed the CL mechanism and found that the two important intermediate species in $NaHCO_3$-H_2O_2 system were the superoxide ion radical ($\bullet O_2^-$) and hydroxide radical (•OH) [17]. The formation of these species (reactive oxygen species) as intermediates was the key to CL emission. To demonstrate the participate of reactive oxygen species in the CL reaction, several scavengers, such as, ascorbic acid and thiourea were added into this proposed CL reaction system. Results indicated that the CL intensity decrease greatly in the presence of these scavengers, which confirmed that these species participated in the CL process. The CL emission is probably due to the formation of excited CdS QDs that could be formed by electron and hole injection.

$$(CdS\ QDs)^* \rightarrow CdS\ QDs + h \quad (6)$$

Analytical Applications

Calibration Curves and Performance Characteristics

In the proposed system, epinephrine quenches the chemiluminescence of the CdS quantum dots in a

Table 6 Sample recovery in urine

Sample	Added (10^{-8}M)	Observed (10^{-8}M)	Recovery (%)	RSD (n=3, %)
1	1	0.987	98.7	3.4
2	5	4.95	99	2.6
3	10	10.3	103	3.2
4	50	52.4	104.8	2.8

Urine was diluted 50-fold in the final assay solutions

concentration dependence that was coincident to the fluorescence quenching described by a Stern–Volmer equation (Eq. 7):

$$I_0/I = 1 + K_{sv}[Q] \quad (7)$$

Where I and I_0 are the chemiluminescence intensities of the CdS QDs at a given epinephrine concentration and in an epinephrine free solution. The $[Q]$ is a epinephrine concentration and K_{sv} is the Stern–Volmer quenching constant. Under the optimum conditions, there is a good linear relationship between the relative chemiluminescence intensity (I_0/I) and the concentration of epinephrine (C) in the range of 5×10^{-9}–1×10^{-6}mol L^{-1} EP with a correlation coefficient (R^2) of 0.9995. The regression equation was $I_0/I = 9\times10^6C + 1.18$. Figure 5, inset, shows a Stern-Volmer quenching curve describing (I_0/I) as a function of epinephrine concentration. K_{sv} is found to be $9\times10^6M^{-1}$. The detection limit (S/N=3) was $5\times10^{-11}molL^{-1}$ epinephrine. From Table 4, it can be seen that the proposed method has a lower detection limit, compared with most of other methods.

Interference Studies

In order to evaluate possible interferences in this system, the effects of some inorganic ions and organic compounds, on the chemiluminescence intensity of the CdS QDs system containing $1.0\times10^{-8}molL^{-1}$ epinephrine were investigated. The tolerance limit was described as the amount of foreign substances which caused relative error less than ±5 % (RSD) in the determination of epinephrine ($1.0\times10^{-8}molL^{-1}$). The tolerable molar concentration ratios with respect to $1.0\times10^{-8}molL^{-1}$ epinephrine were more than 500 for K^+, Na^+, Cl^-, glucose, stearic acid, starch, lactose, bilirubin, urea, thiourea and magnesium stearate, 50 for Ca^{2+}, Zn^{2+}, Ni^{2+}, Mg^{2+}, uric acid and thioglycolic acid, 20 for Cd^{2+} and 10 for Cu^{2+} and Co^{2+}. However, the coexistence of ascorbic acid severely interfered with the determination owing to the strong interaction between CdS QDs and this compound.

Sample Determination and Recovery Tests

To test the applicability of the proposed method, it was applied to the analysis of epinephrine in the epinephrine hydrochloride injection. The samples were diluted appropriately with water before measurement. The results are shown in Table 5. As can be seen, the RSD was 3 % and the recovery of the real samples was 98.8 %, which suggested that there were no significant differences between the compared values, make this new chemiluminescence method applicable to these pharmaceutical formulations. The test of the recovery efficiency for known amounts of epinephrine in human urine is made. This urine sample is obtained from a healthy donor. The urine is diluted 50-fold in the final assay solutions. Results are given in Table 6. The recoveries ranged from 98.7 to 104.8 %, with RSDs of <4 %. It indicated that the proposed method was reliable.

Conclusion

In summary, epinephrine (EP) inhibits strongly the CL intensity of the $NaHCO_3$-H_2O_2-CdS QDs system. Based on this fact, a simple and sensitive CL method for the determination of epinephrine is established, the decrease in the CL signal being proportional to the concentration of epinephrine in the range of 5×10^{-9}–$1\times10^{-6}molL^{-1}$. Moreover, the analytical results of real samples were satisfactory. The proposed method has been applied to the determination of low levels of epinephrine in pharmaceutical products.

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