



## Sensitive Determination of Perphenazine in Pharmaceuticals and Human Serum by Flow Injection Chemiluminescence Method using $[\text{Ru}(\text{phen})_3]^{2+}$ -Ce(IV) System and a Chemometrical Optimization Approach

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Este artigo descreve um método de quimioluminescência (CL) simples, rápido e sensível para a determinação de perfenazina usando sistema de injeção em fluxo (FI). Todas as variáveis que podem afetar a resposta do método CL foram otimizadas empregando planejamento composto central (CCD) para o planejamento experimental e metodologia de superfície de resposta (RSM) para a modelagem. A otimização usando CCD comparativamente ao método univariado mostrou uma melhora significativa na sensibilidade. Sob condições experimentais ótimas, uma ampla região linear foi obtida entre a concentração do analito e a altura do pico, dentro do intervalo de 1,2 a 1.300 ng mL<sup>-1</sup> com coeficiente de correlação de 0,9978. O limite de detecção foi de 0,4 ng mL<sup>-1</sup> (S/N = 3) e o desvio padrão relativo para 6 repetições de uma solução contendo 70,5 ng mL<sup>-1</sup> foi menor que 4%. Esse método foi aplicado com sucesso na quantificação de perfenazina em formulações farmacêuticas e em soro humano com adequadas recuperações (95,3-104,0%). A frequência analítica foi de 100 ± 5 amostras por hora.

This paper describes a simple, rapid and sensitive chemiluminescence (CL) method for the determination of perphenazine by flow injection (FI) system. All variables that can affect the CL response were optimized by employing central composite design (CCD) for the experimental design and response surface methodology (RSM) for the modeling. Optimization by means of CCD method with respect to conventional single factor at a time method showed a significant improvement in the sensitivity. Under the optimum experimental conditions, a wide linear relationship between analyte concentration and peak height was obtained within the range 1.2-1,300 ng mL<sup>-1</sup> with correlation coefficient of 0.9978. The limit of detection was 0.4 ng mL<sup>-1</sup> (S/N = 3) and the relative standard deviation for 6 repeated measurements of a solution containing 70.5 ng mL<sup>-1</sup> was lower than 4%. This method was successfully applied for the quantification of perphenazine in pharmaceutical formulations and human serum with good recoveries (95.3-104.0%). Sample throughput was 100 ± 5 samples per hour.

**Keywords:** flow injection, chemiluminescence, perphenazine, central composite design

### Introduction

Perphenazine belongs to the phenothiazine family of drugs. Because of its neuroleptic and antidepressive actions, it is often prescribed for the treatment of psychotic patients such as schizophrenia and schizoaffective psychoses in order to decrease restlessness, aggressiveness and impulsive behavior. Also it is found to be effectual in the treatment of Parkinson's disease.<sup>1</sup> The monitoring of the perphenazine is important for quality assurance in pharmaceutical industry and for obtaining optimum therapeutic concentrations in body fluids to minimize the risk of toxicity. The

therapeutic range and toxic threshold of perphenazine in serum were reported 4-30 and 50-100 ng mL<sup>-1</sup> respectively.<sup>2</sup> Therefore, it is important to develop simple and sensitive methods for the determination of this drug. A wide variety of analytical techniques are available for the determination of perphenazine in pharmaceutical preparations and biological samples, such as HPLC,<sup>2-6</sup> mass spectrometry,<sup>7-10</sup> spectrophotometry,<sup>11-13</sup> electrochemistry,<sup>14</sup> electrophoresis<sup>15,16</sup> and fluorescence.<sup>17</sup>

Chemiluminescence (CL) is becoming a powerful analytical tool due to its high sensitivity, wide linear dynamic range and simple instrumentation.<sup>18,19</sup> The CL involving  $[\text{Ru}(\text{bpy})_3]^{2+}$  is one of the most interesting series of CL reactions. It involves the oxidation of  $[\text{Ru}(\text{bpy})_3]^{2+}$

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to  $[\text{Ru}(\text{bpy})_3]^{3+}$ , which is following by reduction with an analyte species to produce CL emission.<sup>20,21</sup> Many phenothiazines are tertiary amines which can reduce  $[\text{Ru}(\text{bpy})_3]^{3+}$  and produce intense emission. This behavior has been found in the case of promazine, chlorpromazine, triflupromazine, thioridazine, and trifluoperazine.<sup>22</sup> The luminescence properties of  $[\text{Ru}(\text{phen})_3]^{2+}$  are similar to  $[\text{Ru}(\text{bpy})_3]^{2+}$ , moreover  $[\text{Ru}(\text{phen})_3]^{2+}$  shows greater sensitivity.<sup>23</sup>

CL systems have also been used for the determination of perphenazine. These methods are based on the CL systems including permanganate in sulphuric acid,<sup>24</sup> luminal- $\text{KMnO}_4$ ,<sup>25</sup> luminal-ferricyanide<sup>26,27</sup> and  $\text{Ce}(\text{IV})\text{-HNO}_3$ .<sup>28</sup>

In experimentation, we attempt to monitor the effects of certain inputs or material on the subject matter of interest. The advantages of the experimental design are many. One major advantage is the reduction in the number of experiments, and a second is the possibility of studying the interactions among the various factors. A significant interaction implies that changes in one factor may be dependent on the level of the other factor. If this happens, interpretation of the results has to be done cautiously to avoid inaccurate general statements on the individual factors.<sup>29</sup>

In this work, a sensitive flow-injection CL method has been presented for the determination of perphenazine. It is based on the reaction of the perphenazine with  $[\text{Ru}(\text{phen})_3]^{2+}$  and  $\text{Ce}(\text{IV})$  in sulfuric acid medium. In the method described, the system and chemical variables were optimized by using a univariate procedure, moreover flow rate along with chemical variables were also optimized by using response surface methodology (RSM) and central composite design (CCD). The results from univariate optimization and RSM were compared to each other. In comparison with the above-mentioned CL methods for the analysis of perphenazine, the present method shows greater sensitivity with wider linear dynamic range. This method can be applied for the determination of perphenazine in pharmaceuticals and human serum with satisfactory results.

## Experimental

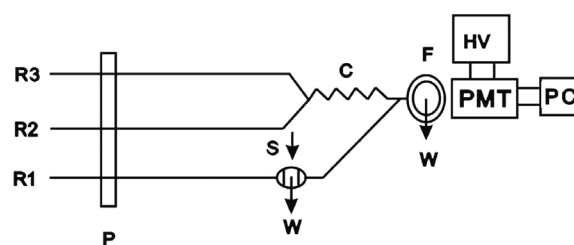
### Reagents and preparation of solutions

All solutions were prepared by using reagent grade chemicals and doubly distilled water. Perphenazine standard solution ( $0.440 \text{ mg mL}^{-1}$ ) was daily prepared by dissolving of 44.0 mg perphenazine hydrochloride (Hallechem Pharma, China) in 100.0 mL water. It was stored in a refrigerator and protected from light. Working solutions were prepared by appropriately diluting

the stock solution when used.  $[\text{Ru}(\text{phen})_3]^{2+}$  solution ( $10.0 \times 10^{-3} \text{ mol L}^{-1}$ ) was prepared by dissolving 0.3640 g of dichlorotris(1,10-phenanthroline) ruthenium(II) hydrate (Sigma-Aldrich, Steinheim, Germany) in water and diluting to the mark in a 50.0 mL volumetric flask.  $\text{Ce}(\text{IV})$  solutions ( $0.2 \times 10^{-3}$ – $4.0 \times 10^{-3} \text{ mol L}^{-1}$ ) were prepared by dissolving proper amount of ceric ammonium nitrate (Riedel-de Haën, Germany) in proper volumes of  $1.0 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$  and diluting to the mark with distilled water in 50.0 mL volumetric flasks. Acetonitrile was HPLC-grade (Caledon, Canada). Perphenazine tablets and ampoules were obtained from Darupakhsh Co., Iran, and serum samples were taken from the Health Center of Isfahan University of Technology (Iran).

### Apparatus

A schematic diagram of the flow system is shown in Figure 1. The CL signal was measured with a CL analyzer with PMT (Model R<sub>212</sub>, Hamamatsu, Japan) and low pass filter whose output was connected to data processing system. A 12-channels peristaltic pump (Desaga, Model PLG, USA) with three silicon rubber tubes (1.0 mm i.d.) was used. PTFE mixing joints and PTFE tubing (1.0 mm i.d.) were used for the connections. The flow cell was U shape with 0.6 mL inner volume and 0.5 cm distance from the PMT. Sample solutions were injected using a six position rotary valve.



**Figure 1.** Schematic diagram of FIA system, R1:  $\text{H}_2\text{O}$ , R2: acidic  $\text{Ce}(\text{IV})$ , R3:  $\text{Ru}(\text{II})$ , P: peristaltic pump, S: injection valve, C: reaction coil, F: flow cell, W: waste, HV: high voltage power supply, PMT: photomultiplier tube, PC: computer.

### Experimental procedure

As shown in Figure 1, channels containing carrier  $\text{H}_2\text{O}$  (R1),  $\text{Ce}(\text{IV})$  solution in sulfuric acid (R2) and  $\text{Ru}(\text{II})$  solution (R3) were pumped at  $3.2 \text{ mL min}^{-1}$  (for each channel) *via* a peristaltic pump. At joint S, an aliquot ( $420 \mu\text{L}$ ) of standard solution of perphenazine was injected into the carrier stream by a sample injection valve. R2 and R3 solutions were mixed through 10 cm reaction coil (silicon tube, 1.0 mm i.d.) and then mixed with

perphenazine exactly in front of PMT to produce peak-like CL emission that is monitored on a computer to smooth the signal and to determine the peak height.

#### Preparation of perphenazine tablets

Ten tablets (2 mg *per* tablet) were weighed and powdered. An accurately weighed portion of the powder, equivalent to 2.0 mg of active ingredient, was transferred into a 100.0 mL calibrated flask containing 50 mL water and the mixture was sonicated for 10 min. Thereafter the volume was adjusted to 100 mL with water and the suspension was filtered. The sample solution was diluted with water, prior to determination step, to obtain the appropriate concentration for analysis.

#### Preparation of perphenazine injection

Contents of five ampoules (5 mg mL<sup>-1</sup>) were transferred into a small beaker. According to manufacturer's specification, an aliquot (1 mL) of the drug was diluted serially to obtain the appropriate concentration for analysis.

#### Procedure for spiked serum

For serum samples only a deproteination process was carried out by using acetonitrile as a sample pretreatment, an extraction procedure was not necessary.<sup>30</sup> An aliquot of standard solution of perphenazine was added to 1.0 mL of serum sample in a centrifuge tube and mixed for 2 min. A volume of 2 mL of acetonitrile was added and centrifuged at 4000 rpm for 15 min. The protein-free supernatant was transferred into a small conical flask and evaporated to dryness under a stream of nitrogen at room temperature. The dry residue was dissolved in 25.0 mL of water. A blank value was determined by treating drug-free serum in the same way. The absolute recovery was determined by comparing the representative peak height of serum sample with the peak height of the standard drug at the same concentration.

## Results and Discussion

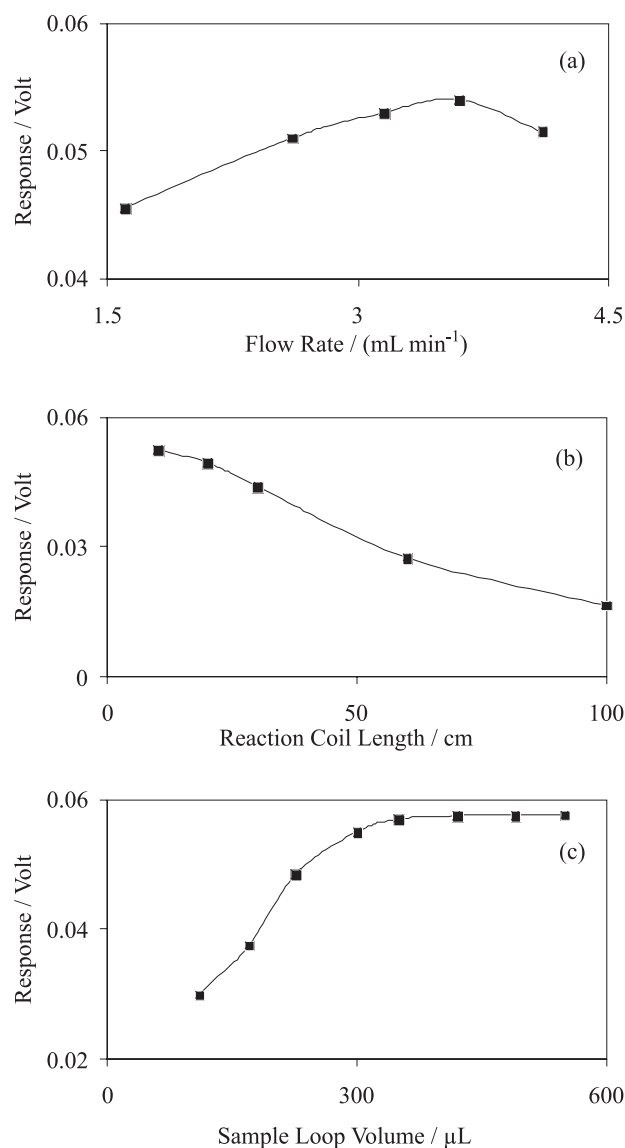
Method is based on rapid reduction of [Ru(phen)<sub>3</sub>]<sup>3+</sup> produced in the reaction between [Ru(phen)<sub>3</sub>]<sup>2+</sup> and acidic Ce(IV) by perphenazine that it produces strong CL.

#### Single factor at a time optimization

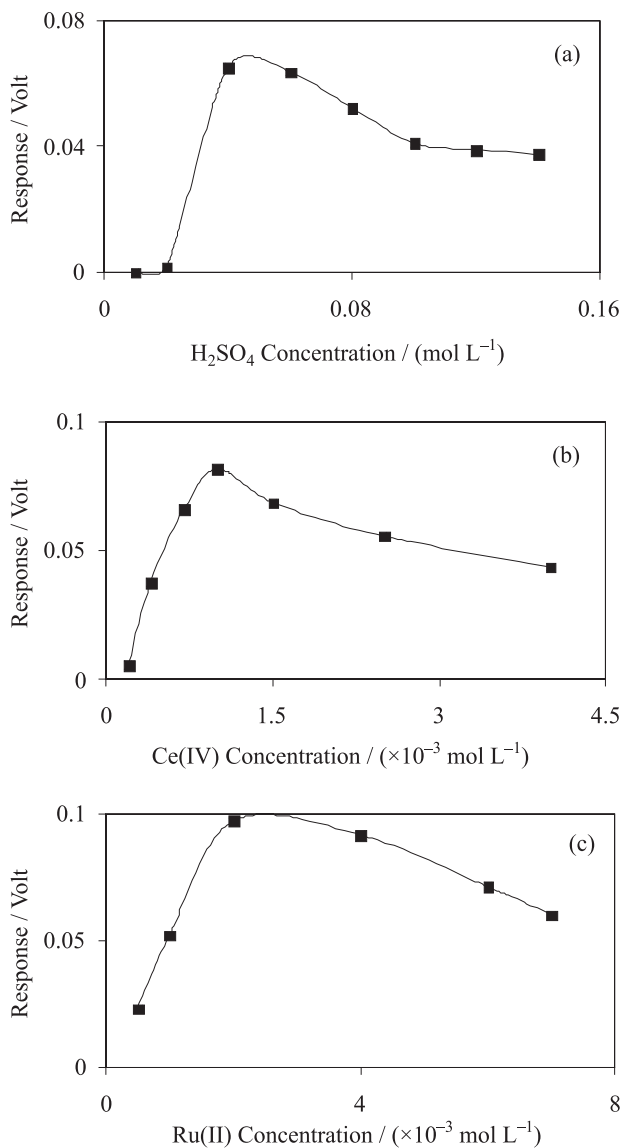
Initial preliminary experiments using the classical single factor at a time method served to detect the variables and

their respective working ranges that have influence on the CL intensity. Optimization results of manifold variables have been shown in Figure 2. For achieving a fast and sensitive method, decreasing the material consumption and preventing of peak broadening, optimum values for the manifold variables were selected as follows: 3.2 mL min<sup>-1</sup> (for each channel), 10 cm and 420 μL for the flow rate, reaction coil length and sample injection volume, respectively.

Single factor at a time optimization of the chemical variables was also performed at optimum condition of manifold variables. Results are shown in Figure 3. According to the results, 1.0×10<sup>-3</sup>, 2.0×10<sup>-3</sup> and 0.04 mol L<sup>-1</sup> were selected as optimum concentrations for Ce(IV), Ru(II) and H<sub>2</sub>SO<sub>4</sub>, respectively.



**Figure 2.** Single factor using a time optimization of manifold variables; a) flow rate, b) reaction coil length, c) sample loop volume. Experiments were performed at 880 ng mL<sup>-1</sup> of perphenazine, 1.0×10<sup>-3</sup> mol L<sup>-1</sup> of Ru(II) and 2.0×10<sup>-3</sup> mol L<sup>-1</sup> of Ce(IV) in 0.08 mol L<sup>-1</sup> of H<sub>2</sub>SO<sub>4</sub>.



**Figure 3.** Single factor at a time optimization of chemical variables; a) Ce(IV), b) Ru(II), c) H<sub>2</sub>SO<sub>4</sub>. Experiments were performed at optimum values of manifold variables, Concentration of perphenazine was 880 ng mL<sup>-1</sup>.

#### Experimental design and response surface modeling

All computations were performed using Matlab (The Math-Works Inc., Natick, MA, USA).

After detecting respective working ranges of the variables, three chemical variables including concentrations of Ru(II), Ce(IV) and H<sub>2</sub>SO<sub>4</sub> along with flow rate were studied using CCD. As it can be seen in Figure 2, some manifold variables (injection volume and reaction coil) almost have linear changes in the range which they were studied. Therefore optimum conditions of these manifolds obtained by single factor at a time method were used in the multivariate optimization of chemical variables (optimum conditions of these two manifolds supposed to be the same

in both single factor at a time and multivariate methods).

In the optimization step, concentration of perphenazine was 880 ng mL<sup>-1</sup>, similarly to that used in the single factor at a time method.

CCD including four factors and five levels for each factor ( $\alpha = 2$ ) was designed for the experiments. The coded levels of the variables together with their real experimental values are given in Table 1 and experiments are listed in Table 2.

**Table 1.** Factor levels and their respective actual values used in the CCD

Coded levels	Ce(IV)/ ( $\times 10^{-3}$ mol L <sup>-1</sup> )	Ru(II)/ ( $\times 10^{-3}$ mol L <sup>-1</sup> )	H <sub>2</sub> SO <sub>4</sub> / (mol L <sup>-1</sup> )	Flow Rate/ (mL min <sup>-1</sup> )
- $\alpha$	0.5	0.5	0.03	1.6
-1	1.5	1.0	0.04	2.1
0	2.5	1.5	0.05	2.6
+1	3.5	2.0	0.06	3.1
+ $\alpha$	4.5	2.5	0.07	3.6

In order to describe the way in which the variables are related and the way in which they influence the response, response surface methodology (RSM)<sup>24</sup> was used to assemble the model. Therefore, the data obtained from the set of conditions employed by the CCD were fit to the following parametric equation (full second order polynomial).

$$I_{CL} = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_1X_2 + b_6X_1X_3 + b_7X_1X_4 + b_8X_2X_3 + b_9X_2X_4 + b_{10}X_3X_4 + b_{11}X_1^2 + b_{12}X_2^2 + b_{13}X_3^2 + b_{14}X_4^2 \quad (1)$$

Where  $I_{CL}$  (CL intensity) is the response,  $b_0$  is the intercept,  $X_s$  are the four variables,  $b_1$ - $b_4$  are the linear parameters,  $b_5$ - $b_{10}$  represents the interaction parameters and  $b_{11}$ - $b_{14}$  are the quadratic parameters. Table 3 gives the estimates of 15 parameters, contained in equation 1 obtained by the matrix least squares.

The significance of regression was tested by the  $p$  value and found to be highly significant. Analysis of variance (ANOVA) of the results showed that Ce(IV) and H<sub>2</sub>SO<sub>4</sub> concentrations along with interaction between them were important terms and they had a statistically significant effect on the CL Intensity ( $p < 0.05$  at a 95% confidence level).

Analysis of the residuals from the regression model and the lack of fit test revealed that the second order polynomial model, tentatively assumed, would be an adequate description of the surface over the region studied. The operational optimum was determined using a grid method on equation 1, while gradually varying factor levels from -2 to +2. The levels corresponding to the maximum response

**Table 2.** Design matrix designed by CCD and responses obtained for each experiment

Random Run No.	Ce ( $X_1$ )/( $\times 10^{-3}$ mol L $^{-1}$ )	Ru ( $X_2$ )/( $\times 10^{-3}$ mol L $^{-1}$ )	H $_2$ SO $_4$ ( $X_3$ )/(mol L $^{-1}$ )	Flow rate ( $X_4$ )/(mL min $^{-1}$ )	Response/Volt
8	2.5	1.5	0.03	2.6	0.020
11	1.5	1.0	0.04	2.1	0.219
16	1.5	1.0	0.04	3.1	0.263
4	3.5	1.0	0.04	3.1	0.191
19	3.5	1.0	0.04	2.1	0.172
7	1.5	2.0	0.04	2.1	0.161
18	1.5	2.0	0.04	3.1	0.141
14	3.5	2.0	0.04	3.1	0.119
23	3.5	2.0	0.04	2.1	0.122
25	2.5	0.5	0.05	2.6	0.151
12	0.5	1.5	0.05	2.6	0.079
1	2.5	1.5	0.05	2.6	0.376
5	2.5	1.5	0.05	3.6	0.420
6	2.5	1.5	0.05	2.6	0.390
15	2.5	1.5	0.05	2.6	0.395
17	2.5	1.5	0.05	2.6	0.387
28	2.5	1.5	0.05	1.6	0.282
26	4.5	1.5	0.05	2.6	0.303
20	2.5	2.5	0.05	2.6	0.270
3	1.5	1.0	0.06	3.1	0.480
10	1.5	1.0	0.06	2.1	0.446
2	3.5	1.0	0.06	2.1	0.514
27	3.5	1.0	0.06	3.1	0.602
9	1.5	2.0	0.06	2.1	0.358
22	1.5	2.0	0.06	3.1	0.418
21	3.5	2.0	0.06	2.1	0.864
24	3.5	2.0	0.06	3.1	0.931
13	2.5	1.5	0.07	2.6	0.499

**Table 3.** Regression results and analysis of variance of RSM

Source	Parameter	Parameter Estimate	P Value
Intercept	$b_0$	0.773	0.0000
Ce(IV) A	$b_1$	0.123	0.0172
Ru(II) B	$b_2$	0.038	0.4087
H $_2$ SO $_4$ C	$b_3$	0.349	< 0.0001
Flow D	$b_4$	0.047	0.3192
AB	$b_5$	0.110	0.0660
AC	$b_6$	0.174	0.0074
AD	$b_7$	0.006	0.9165
BC	$b_8$	0.105	0.0798
BD	$b_9$	-0.011	0.8480
CD	$b_{10}$	0.027	0.6316
AA	$b_{11}$	-0.057	0.2244
BB	$b_{12}$	-0.048	0.3091
CC	$b_{13}$	-0.023	0.6129
DD	$b_{14}$	0.023	0.6250
Model			0.0009
R $^2$	0.92		
Adjusted R $^2$	0.85		

Factors in italic were found to be statically significant after ANOVA analysis of the results ( $p$  Value < 0.05 at a 95% confidence level).

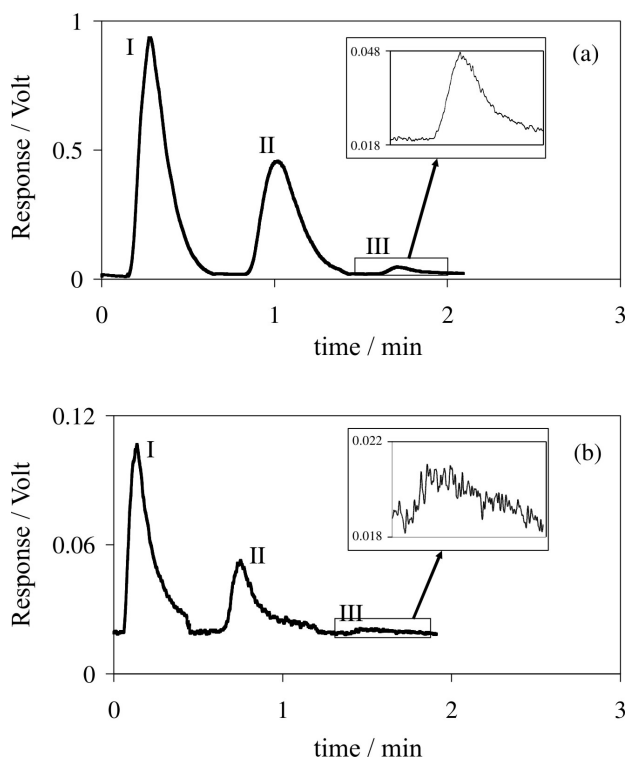
selected as optimum levels.<sup>24</sup> The optimum levels and their respective actual values are shown in Table 4.

Typical CL profiles for three different concentrations including 880.0, 440.0 and 44.0 ng mL $^{-1}$  of perphenazine at optimum conditions obtained using RSM (Figure 4a) and optimum conditions obtained using single factor at a time method (Figure 4b) were compared to each other. As it can be seen in Figure 4, optimization using RSM method with respect to conventional single factor at a time method shows a significant improvement in the sensitivity (approximately nine times).

The results obtained by experimental design procedure were better than single factor at a time method. The main reason could be due to interaction between variables. The portion of these interactions in multivariate optimization methods could be calculated and employed in optimization of variables. But in the univariate optimization of variables, effect of these interactions could not be determined. Therefore optimum conditions obtained by single factor at a time method will be different from real optimum conditions when interactions between variables are important.

**Table 4.** The optimum levels and their respective actual values obtained by response surface modeling

Variable	Coded Level	Actual Level
Ce(IV)	+1.48	$3.98 \times 10^{-3} \text{ mol L}^{-1}$
Ru(II)	+0.92	$1.96 \times 10^{-3} \text{ mol L}^{-1}$
H <sub>2</sub> SO <sub>4</sub>	+2.00	$0.07 \text{ mol L}^{-1}$
Flow Rate	+0.76	$3.22 \text{ mL min}^{-1}$

**Figure 4.** Comparison between optimizations using conventional single factor at a time and RSM methods; a) Optimization using RSM, b) Optimization using single factor at a time; I: 880.0, II: 440.0, III: 44.0 ng mL<sup>-1</sup>.

### Analytical features

The results show that a relatively wide linear dynamic range is obtained in the range of 1.2-1,300 ng mL<sup>-1</sup> which its linear equation was:  $I_{CL} = 0.0011(\pm 0.0000) C_{\text{Perphenazine}} - 0.0003 (\pm 0.0001)$ ,

( $r^2 = 0.9978$ ), in that  $C_{\text{Perphenazine}}$  is concentration of perphenazine (ng mL<sup>-1</sup>). The limit of detection was 0.4 ng mL<sup>-1</sup> (S/N = 3) and relative standard deviation for 6 replicates determination of a solution containing 70.5 ng mL<sup>-1</sup> of perphenazine was < 4%. Sample throughput was  $100 \pm 5$  samples per hour.

### Influence of interfering substances

In order to validate based on the possible analytical application of the method, interference effect of some common ions, excipients in pharmaceutical preparation and some amino acids commonly present in human serum were studied by recovering 44.0 ng mL<sup>-1</sup> of perphenazine in presence of each substance. The tolerance of each substance was taken as the largest amount yielding an error lower than  $3\sigma$  in the analytical signal of 44.0 ng mL<sup>-1</sup> perphenazine ( $\sigma$  is the standard deviation in signal for 10 times replication of 44.0 ng mL<sup>-1</sup>). It was found that the presence of the common excipients of pharmaceuticals and some amino acids did not interfere in the determination of the studied drug, indicating high accuracy and suitability for determination of perphenazine in human serum and quality assurance in drug formulations. Results are shown in Table 5.

### Application

In order to evaluate the applicability of the proposed method, two perphenazine drugs and spiked human serum were analyzed to determine their perphenazine contents. Table 6 shows the results of the recovery studies of perphenazine from drugs and spiked serum. Results show good reproducibility and accuracy of the method. The proposed method was further applied to the analysis of certain dosage forms containing perphenazine. Results in Table 7 are in agreement with those obtained by the official method.<sup>31</sup> Statistical analysis of the results using student *t*-test and the variance ratio *F*-test showed no significant difference between the performance of the two methods based on accuracy and precision.

**Table 5.** Effect of foreign substances on the determination of 44 ng mL<sup>-1</sup> perphenazine

Substance	Concentration ratio of substance to perphenazine
Sucrose, Glucose, Fructose, Saccharin, starch, Serine, Valine, Threonine, Cl <sup>-</sup> , Lactose, Leucine, K <sup>+</sup> , Urea	1000
Alanine, Glycine, Proline, Na <sup>+</sup> , HCO <sub>3</sub> <sup>-</sup> , CO <sub>3</sub> <sup>2-</sup> , PO <sub>4</sub> <sup>3-</sup> , Cu(II), Cr(III), Cd(II)	100
Aspartic acid, Tyrosine, Ca(II), Fe(II), Mg(II)	50
Tryptophane, Cysteine	10
Chlorpromazine, Fluphenazine, Promazine	5

**Table 6.** Determination of perphenazine in commercial formulations and human serum

Sample	Sample No.	Added/(ng mL <sup>-1</sup> )	Total Found/(ng mL <sup>-1</sup> )	Recovered/(%)
Perphenazine Tablet	1	-	31.5 ± 1.0	-
	2	40.0	71.2 ± 2.1	99.3
	3	80.0	114.6 ± 1.4	103.9
	4	160.0	197.7 ± 6.3	103.9
Perphenazine Injection	1	-	40.1 ± 1.4	-
	2	40.0	80.0 ± 2.2	99.8
	3	80.0	118.3 ± 0.9	97.8
	4	160.0	200.7 ± 4.2	100.4
Human Serum	1	10.0	10.4 ± 0.4	104.0
	2	40.0	38.7 ± 1.4	96.8
	3	80.0	79.3 ± 3.1	99.1
	4	160.0	152.5 ± 6.7	95.3

**Table 7.** Analysis of some dosage forms containing perphenazine using the proposed and official methods

Drug	Claimed	Recovery	
		Proposed Method <sup>a</sup>	Official Method <sup>a,31</sup>
Perphenazine Injection/(mg mL <sup>-1</sup> )	5	5.01 ± 0.17	5.08 ± 0.07
Perphenazine Tablet/mg	2	1.97 ± 0.06	1.99 ± 0.03

<sup>a</sup>mean values of four replication.

### Response characteristics

In the Table 8, response characteristics of the proposed method are compared with recently reported methods. In comparison with CL methods,<sup>24-26,28</sup> the proposed method is sensitive with a wide linear dynamic range, moreover it has been applied for the quantification of perphenazine in human serum and pharmaceuticals. Some of the sensitive methods in Table 8, besides chemical information for perphenazine, provide multianalyte information about related species, compounds and metabolites presented in the sample. However they have a complex, time consuming and expensive instrumentation and some of them use a preconcentration method before the measurement.<sup>4-10,16,32</sup> The proposed system is an easy, cheap and fast analytical tool for obtaining of preliminary chemical information about perphenazine, prior the use of more complex instrumental techniques.

### Possible CL mechanism

Some CL pathways might be proposed for the [Ru(phen)<sub>3</sub>]<sup>2+</sup> - Ce(IV) - perphenazine CL system, involving

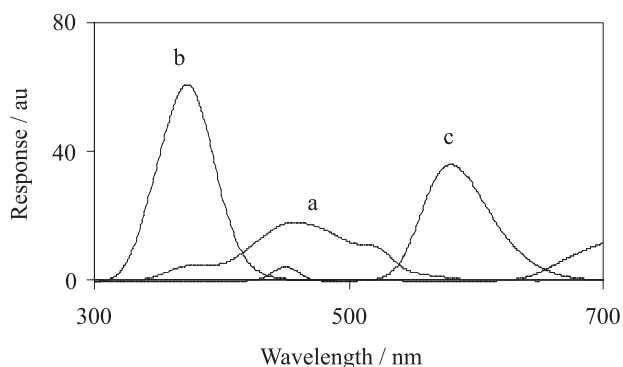
the formation of Ce(III)\*, oxidation products in excited state (such as sulphone derivative) and [Ru(phen)<sub>3</sub>]<sup>2+\*</sup>. To explore the possible CL mechanism, some experiments performed and following results were obtained. (i) A weak CL response was recorded when [Ru(phen)<sub>3</sub>]<sup>2+</sup> solution was mixed with acidic Ce(IV) solution. Enhancement in CL response was detected when perphenazine solution was injected into the mixed solution of [Ru(phen)<sub>3</sub>]<sup>2+</sup> and acidic Ce(IV). (ii) Fluorescence spectrum of Perphenazine ( $\lambda_{ex}$  = 253 nm) was scanned using spectrofluorometer (Jasco, model FP-750) using batch mode. Perphenazine had a maximum emission wavelength at 460 nm (Figure 5a). This peak was disappeared when Ce(IV) solution was added into the cuvette and new peak appeared at 375 nm (Figure 5b). These are due to oxidation of perphenazine and formation of Ce(III) that is a well known fluorescent ion.<sup>35</sup> Fluorescence emission spectrum of [Ru(phen)<sub>3</sub>]<sup>2+</sup> ( $\lambda_{ex}$  = 450 nm), had a maximum at 582 nm (Figure 5c). (iii) CL spectra of mixtures including Ce(IV)-perphenazine (Figure 6a), Ce(IV)-[Ru(phen)<sub>3</sub>]<sup>2+</sup> (Figure 6b) and Ce(IV)-[Ru(phen)<sub>3</sub>]<sup>2+</sup>-perphenazine (Figure 6c) were obtained using spectrofluorometer (Jasco, model FP-750). No detectable CL response obtained for the first mixture. This suggests that oxidation products and Ce(III)\* are not main emitters. Moreover both spectra of second and third mixtures had the same maximum emission wavelength at 581 nm which is same as maximum fluorescence emission of [Ru(phen)<sub>3</sub>]<sup>2+</sup>. This indicated that the CL spectra were independent of perphenazine and the emitter is [Ru(phen)<sub>3</sub>]<sup>2+\*</sup>.

Perphenazine is a tertiary amine and from previous studies, the oxidation of tertiary amines is understood to produce a short-lived radical cation. The  $\alpha$ -carbon is then deprotonated, yielding a strongly reducing intermediate.

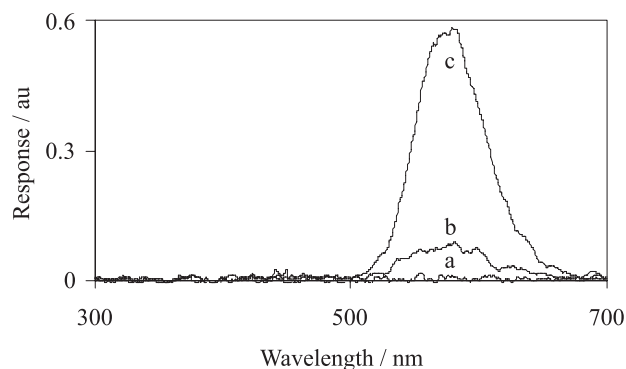
**Table 8.** Comparison between recently reported methods and proposed method

Method	Sample	Linear Range/(ng mL <sup>-1</sup> )	Detection Limit/(ng mL <sup>-1</sup> )	Ref.
HPLC <sup>a</sup>	Serum	Not Reported	0.5	5
	Serum	4-500	4	2
	No Sample	Not Reported	2.6	3
	Sheep Plasma	1.00-100.00	0.04	32
SPE <sup>b</sup> -HPLC	Serum	1.0-100.0	0.4	6
	Serum	1.0-10.0	1.3	4
HPLC-MS-MS <sup>c</sup>	Serum	1.0-20.0	0.3	8
HPLC-MS	Urine, Blood	5.0-500.0	1.7	7
GC <sup>d</sup> -MS	Urine	4.0-100.0	1.2	9
SPME <sup>e</sup> -LC-MS-MS	Urine	0.10-50.00	0.01	10
Spectrophotometry	Tablet, Injection	(4.4-70.5)×10 <sup>3</sup>	2.3×10 <sup>3</sup>	11
	Tablet	(4.0-60.0)×10 <sup>3</sup>	1.3×10 <sup>3</sup>	31
	Tablet	(3.7-18.2)×10 <sup>3</sup>	981.0	13
	Tablet	(5.0-50.0)×10 <sup>3</sup>	1.7×10 <sup>3</sup>	33
Titration	Tablet, injection	(2.2-4.4)×10 <sup>4</sup>	Not Reported	34
Fluorescence	No Sample	10-200	10	17
CZE <sup>f</sup>	Urine	44.1-44050.0	22.0	15
SPE-CZE	Serum, Injection	7-250	3	16
Electrochemistry	Tablet	2.6-220.3	Not Reported	14
CL <sup>g</sup>	No Sample	4.0-3000.0	0.7	25
	Tablet	(0.1-70.0)×10 <sup>3</sup>	80.0	28
	Urine	50-100000	20	26
	Synthetic	(5-35) ×10 <sup>4</sup>	1×10 <sup>4</sup>	24
	Tablet, Injection, Serum	1.2-1300.0	0.4	Proposed Method

<sup>a</sup>High performance liquid chromatography; <sup>b</sup>Solid phase extraction; <sup>c</sup>Mass spectrometry; <sup>d</sup>Gas chromatography; <sup>e</sup>Solid-phase micro extraction; <sup>f</sup>Capillary zone electrophoresis; <sup>g</sup>Chemiluminescence.



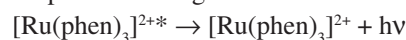
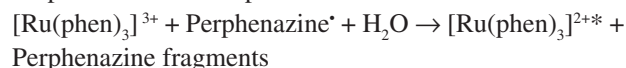
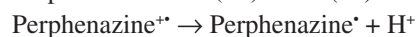
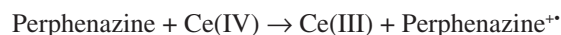
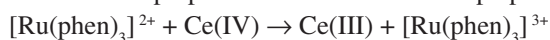
**Figure 5.** The fluorescence spectrum of a) Perphenazine (20.0 µg mL<sup>-1</sup>) at  $\lambda_{ex} = 253$  nm, b) Perphenazine (2 mL 20.0 µg mL<sup>-1</sup>) and Ce(IV) (200 µL (3.0×10<sup>-3</sup> mol L<sup>-1</sup> in 0.08 mol L<sup>-1</sup> of H<sub>2</sub>SO<sub>4</sub>)), at  $\lambda_{ex} = 253$  nm, c) [Ru(phen)<sub>3</sub>]<sup>2+</sup> (1.0×10<sup>-3</sup> mol L<sup>-1</sup>), at  $\lambda_{ex} = 450$  nm. The weak peak at 450 nm is due to Rayleigh scattering of [Ru(phen)<sub>3</sub>]<sup>2+</sup>.



**Figure 6.** The CL spectrum of a) Ce(IV) – perphenazine, b) Ce(IV) – [Ru(phen)<sub>3</sub>]<sup>2+</sup>, c) Ce(IV) – [Ru(phen)<sub>3</sub>]<sup>2+</sup> – perphenazine. Conditions: a) 2 mL perphenazine (20.0 µg mL<sup>-1</sup>) and 200 µL Ce(IV) (3.0×10<sup>-3</sup> mol L<sup>-1</sup> in 0.08 mol L<sup>-1</sup> of H<sub>2</sub>SO<sub>4</sub>), b) 2 mL [Ru(phen)<sub>3</sub>]<sup>2+</sup> (1.0×10<sup>-3</sup> mol L<sup>-1</sup>) and 200 µL Ce(IV) (3.0×10<sup>-3</sup> mol L<sup>-1</sup> in 0.08 mol L<sup>-1</sup> of H<sub>2</sub>SO<sub>4</sub>), c) 2 mL [Ru(phen)<sub>3</sub>]<sup>2+</sup> (1.0×10<sup>-3</sup> mol L<sup>-1</sup>), 200 µL perphenazine (20 µg mL<sup>-1</sup>) and 200 µL Ce(IV) (3.0×10<sup>-3</sup> mol L<sup>-1</sup> in 0.08 mol L<sup>-1</sup> of H<sub>2</sub>SO<sub>4</sub>).

This reduces the [Ru(phen)<sub>3</sub>]<sup>3+</sup> (produced by oxidant) to the excited state that subsequently emits light.<sup>30,36-38</sup>

According to the above discussion, the following mechanism is proposed for the CL reaction of perphenazine.





## Conclusions

A new CL method was proposed for the flow injection quantification of perphenazine. The method is simple, sensitive and rapid with wide linear dynamic range between 1.2 and 1,300 ng mL<sup>-1</sup> and has been used to determine perphenazine in pharmaceuticals and human serum. Moreover some common sugars, amino acids and ions did not present any significant interference effects in the quantification of perphenazine. Therefore high accuracy and precision determination of perphenazine could be performed in human serum and drug formulations by the proposed method. Because of interaction between CL variables, experimental design is useful and important part in CL detections but it simply disregarded in many CL measurements. One future trend might be the combination of the proposed CL system ([Ru(phen)<sub>3</sub>]<sup>2+</sup> and acidic Ce(IV)) which was used previously for the determination of some other phenothiazines,<sup>22</sup> with liquid chromatography equipment, developing a proper technique for the determination of phenothiazines in various matrixes and pharmaceuticals.

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