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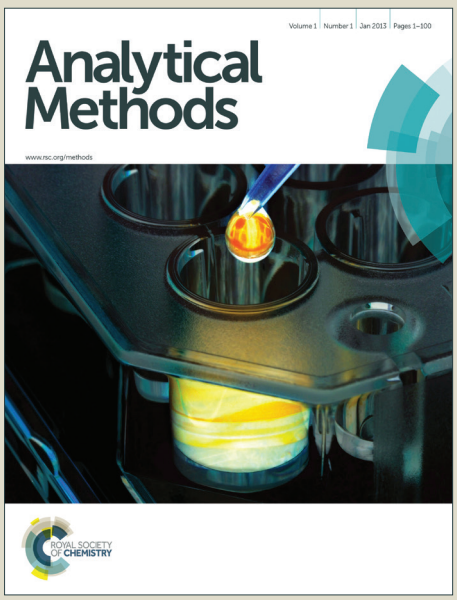

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ARTICLE TYPE

Sensitive Determination of Aripiprazole Using Chemiluminescence Reaction of tris(1,10 phenanthroline)ruthenium(II) with Acidic Ce(IV)

Ali Mokhtari

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Aripiprazole (ARP) is an atypical antipsychotic medication used for the treatment of schizophrenia and schizoaffective disorders. In this study, a new method using chemiluminescence (CL) of tris(1,10-phenanthroline)-ruthenium(II), $Ru(phen)_3^{2+}$, was developed for the rapid and sensitive determination of ARP in pharmaceuticals and human plasma. The method is based on that the weak chemiluminescence produced in the reaction of $Ru(phen)_3^{2+}$ and acidic Ce(IV), enhances in the presence of ARP. Under the selected experimental conditions, calibration curves were linear from 1.8 to 18.0 ng mL^{-1} (r^2 = 0.9951) and from 18 to 35900 ng mL^{-1} (r^2 = 0.9987). The limit of detection (LOD) was 0.9 ng mL^{-1} (S/N=3). In the method proposed, LOD was about 100 times lower than the therapeutic concentration of ARP. The percent relative standard deviations (%RSDs) for 11 repeated measurements of 180 and 720 ng mL^{-1} of ARP were 4.5 and 5.2 %, respectively. The sampling rate for analysis was 70 samples per hour. The proposed method was successfully applied to the assay of commercial tablets containing the drug, and the results were in accordance with those obtained with reference method. The method was further applied to the determination of the drug in plasma samples. The possible CL reaction mechanism was also discussed briefly.

Introduction

Aripiprazole (ARP), a quinolinone derivative, is an atypical antipsychotic and antidepressant used in the treatment of schizophrenia, bipolar disorder and clinical depression [1]. Chemical structure of ARP is shown in fig. 1.

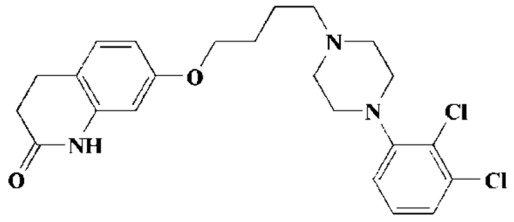

Fig. 1 Chemical structure of ARP

ARP represents a well-tolerated and effective addition to the antipsychotic armamentarium. It acts as a potent partial agonist at dopamine D2 receptors and serotonin 5-HT1A receptors [2]. The monitoring of the ARP is important for quality assurance in pharmaceutical industry and for obtaining optimum therapeutic concentrations in body fluids to minimize the risk of toxicity. Therapeutic concentration for ARP is in the range 100-450 ng mL^{-1} [3-5]. Therefore, it is important to develop simple and sensitive methods for the determination of this drug. A wide variety of methods are available for the determination of ARP in pharmaceutical preparations and biological samples, such as chromatography [6-17], mass spectrometry [18-27], electrophoresis [28, 29], spectrophotometry [30-36] and electrochemistry [37, 38].

Chemiluminescence (CL) is an attractive means of detection because it presents low detection limits, a wide linear working range and uses relatively simple instrumentation. For these reasons, CL has received much attention in various fields, especially combination with separation methods, for analysis of drugs in biological samples [39-45]. CL relying on the effects related to the chemical reaction only, i.e. without the need of external energy supply, has been found to be more advantageous than other luminescence methods [46]. Ruthenium(II) complexes, such as tris(2,2-bipyridyl)-ruthenium(II), $Ru(bpy)_3^{2+}$, and tris(1,10-phenanthroline)-ruthenium(II), $Ru(phen)_3^{2+}$, are among the reagents most frequently used for the generation of CL [47]. Compared to $Ru(bpy)_3^{2+}$, $Ru(phen)_3^{2+}$ exhibits higher enhancement and sensitivity [48].

Different oxidation methods could be used for oxidizing the Ru(II) complexes and the studied substances, such as electrochemistry [49], $K_2S_2O_8$ [50], KIO_4 [51], $KMnO_4$ [52] and PbO_2 [53]. Ce(IV) is one of the most extensively used reagents in organic chemistry. The reasons for its general acceptance as a one-electron oxidant may be attributed to its large reduction potential value of +1.61 V vs. NHE (normal hydrogen electrode), low toxicity, ease of handling, experimental simplicity, and solubility in a number of organic solvents [54]. Ce(IV) in acidic medium could be used as a CL reagent alone [55-57] or along with a sensitizer such as rhodamine 6G [58, 59], rhodamine B [60], Quinine [61], sodium sulphite [62] and $Ru(bpy)_3^{2+}$ [63-65].

In this method, CL light emission was generated during

oxidation of $Ru(phen)_3^{2+}$ by Ce(IV) in sulfuric acid medium and the emission intensity was greatly enhanced in the presence of ARP. To our best knowledge, this is the first CL method proposed for the determination of ARP up to now. The proposed method was successfully used for the quantification of ARP in tablets and human plasma samples.

Experimental

All the experiments involving human subjects were approved by the Golestan University's committee and they were performed in compliance with the relevant laws and institutional guidelines. Moreover, written consent was obtained from any human subjects prior to the experiment.

Materials and preparation of solutions

All the solutions were prepared by using reagent grade chemicals and doubly distilled water. Acetonitrile was HPLC-grade (Caledon, Canada). ARP standard solution (1.0×10^{-3} mol L^{-1}, 450.0 μg mL^{-1}) was daily prepared by dissolving of 45.0 mg ARP (Tofigh Daru, Iran) in a mixture including, 3.0 mL H_2O, 600 μL concentrated HCl (Merk) and 8.0 mL acetone (Merck) and then diluted to the mark with water in a 100.0 mL volumetric flask. Working solutions were prepared by appropriate diluting the stock solution when used. $Ru(phen)_3^{2+}$solution (1.0×10^{-2} mol L^{-1}) was prepared by dissolving 0.3640 g of dichlorotris (1,10-phenanthroline)-ruthenium(II) hydrate (Sigma-Aldrich, Steinheim, Germany) in 50.0 mL water. Ce(IV) solutions (1.0×10^{-3}-9.0×10^{-3} mol L^{-1}) were prepared by dissolving of calculated amount of ceric ammonium nitrate (Riedel-de Haën, Germany) in proper volumes of H_2SO_4 1.0 mol L^{-1} and diluting to the mark with distilled water in 50.0 mL volumetric flasks. Plasma samples were taken from the health center of Gorgan (Iran). ARP tablets were purchased from local drugstores.

Apparatus

CL analysis was applied using a 0.50 cm light path length quartz cell. The CL signal was measured with a laboratory-built CL analyzer with PMT (Hammamatso, model R_{212}, Japan) which its output after amplifying was connected to a PC via a 16 bit analog to digital (A to D) converter. The light emitted by the CL reaction was detected with no wavelength discrimination. A schematic block diagram of the used instruments is shown in fig. 2.

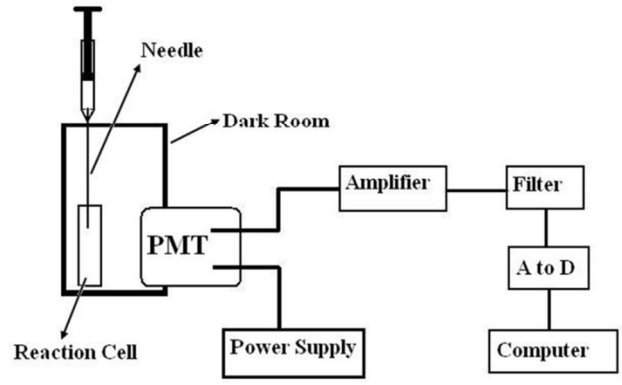

Fig. 2 Schematic block diagram of the CL instrument

General procedure

An aliquot (200 μL) of standard solution consisting of ARP with 400 μL of 2.0×10^{-3} mol L^{-1} of $Ru(phen)_3^{2+}$ were transferred into the 0.50 cm path light length quartz cell. Then, the cell was placed at its location in front of PMT and the program was started. After a few seconds, 400 μL of acidic Ce(IV) was injected into the cell by a microsyringe and the peak-like CL emission was recorded by a computer (with interval times of 100 ms). Those data information were collected into Excel software. Maximum CL response of ARP appeared about 1-1.5 seconds after injection of Ce(IV) solution. For obtaining the analytical signal, response from the blank was subtracted from maximum peak height of each sample.

Preparation of tablets

Ten tablets of the drug were weighed and powdered. An accurately weighed portion of the powder, including active ingredients equivalent to one tablet dosage, was transferred into a 250.0 mL volumetric flask containing 50.0 mL H_2O, 200 μL concentrated HCl and 3.0 mL acetone. The mixture was sonicated for 10 minutes. Then the volume was adjusted to 250.0 mL with water and the suspension was filtered. An appropriate volume of the sample solution was further diluted with water so that the final ARP concentration was in the working range.

Procedure for plasma samples

Only a deproteination process was carried out by using acetonitrile as a sample pretreatment and extraction procedure was not necessary [66]. The standard addition method was used for the determination of ARP in the plasma samples. Therefore each time, 0.4 mL of plasma sample was transferred into a centrifuge tube including 2 mL of acetonitrile and the mixture centrifuged at 4000 r/min 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 transferred into a 25.0 mL flask using double distilled water, then the standard solution was added into the flask and the mixture was diluted to the mark.

Results and discussion

Kinetic curve of the CL reaction

Typical CL profiles for five different concentrations including 1.8, 180, 1430, 3590 and 14300 ng mL^{-1} of APR at optimum conditions are shown in fig. 3

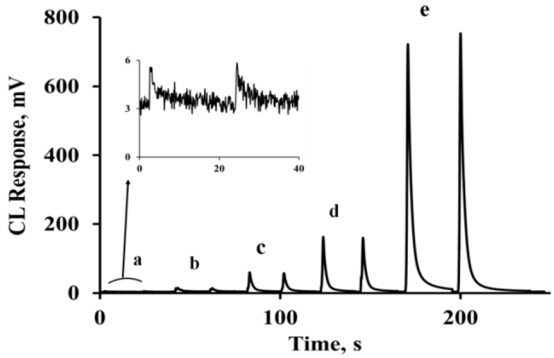

Fig. 3 Typical CL profiles for five different concentrations of APR including 1.8, 180, 1430, 3590 and 14300 ng mL^{-1}

CL profiles demonstrated that the CL reaction was very quick. It only took 1-1.5 s to achieve the maximum peak, compared with 15-30 s for the signal to decline to base.

Optimization of chemical variables

Classical single factor at a time method served to detect the variables and their respective working ranges that have influence on the CL intensity. Influence of $Ru(phen)_3^{2+}$, Ce(IV) and H_2SO_4 concentrations on the sensitivity were investigated in presence of 3.6 µg mL^{-1} ARP. The results have been shown in fig. 4 to 6. According to the results, 3.0×10^{-3} mol L^{-1}, and 0.07 mol L^{-1} were selected as optimum concentrations for Ce(IV) and H_2SO_4, respectively. For decreasing the material consumption, concentration of 2.0×10^{-3} mol L^{-1} was selected as optimum concentration of $Ru(phen)_3^{2+}$. For comparing the type of oxidant, a $KMnO_4$ solution with concentration of 3×10^{-3} mol L^{-1} in 0.1 mol L^{-1} H_2SO_4 was also investigated. In this experiment Ce(IV) solution produced 2.5 to 3 times CL intensity more than $KMnO_4$ solution.

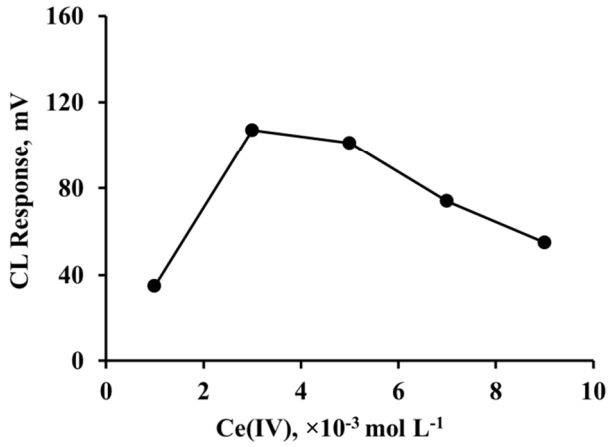

Fig. 4 Influence of Ce(IV) concentration on the CL signal at 2×10^{-3} mol L^{-1} $Ru(phen)_3^{2+}$, 0.10 mol L^{-1} H_2SO_4 and 3.6 µL mL^{-1} of ARP

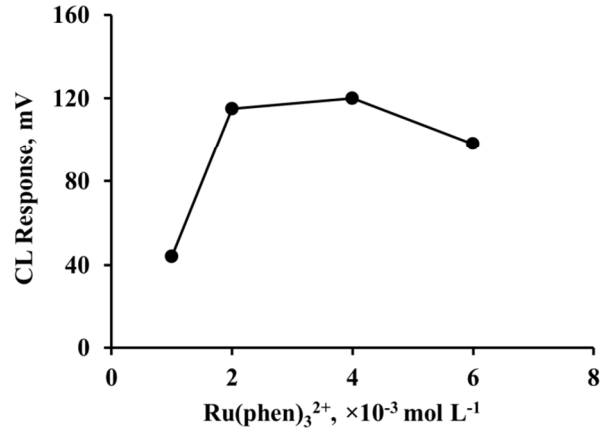

Fig. 5 Influence of $Ru(phen)_3^{2+}$ concentration on the sensitivity at 3.0×10^{-3} mol L^{-1} Ce(IV), 0.10 mol L^{-1} H_2SO_4 and 3.6 µL mL^{-1} of ARP

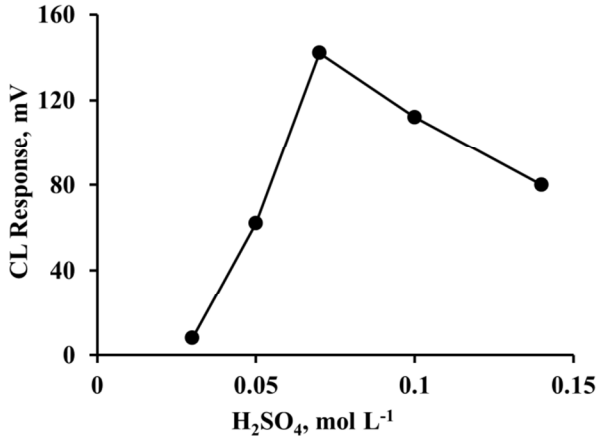

Fig. 6 Influence of H_2SO_4 concentration on the sensitivity at 2×10^{-3} mol L^{-1} $Ru(phen)_3^{2+}$, 3.0×10^{-3} mol L^{-1} Ce(IV) and 3.6 µL mL^{-1} of ARP

Analytical features

Under optimum conditions, a long series of standard solutions of ARP were subjected to the optimized CL method for the purpose of calibration. CL response was found to be linear in the concentration ranges of 1.8-18.0 ng mL^{-1} and 18-35900 ng mL^{-1}. The correlation equation between CL intensity and concentration of ARP in linear ranges were: $I_{CL} = 122.37C_{ARP} + 0.94$ (R^2 = 0.9951) and $I_{CL} = 47.71C_{ARP} - 2.81$ (R^2 = 0.9987), respectively (where I_{CL} is CL intensity (mV) and C_{ARP} is ARP concentration (µg mL^{-1})).

The limit of detection (LOD) was calculated as 3σ/m where σ is the standard deviation existing in 10 times determination of the blank response and m is slope of the lower calibration curve (1.8-18.0 ng mL^{-1}). The LOD obtained was 0.9 ng mL^{-1}, indicating good detectability (because it is at least 100 times lower than the therapeutic concentration of ARP). The reproducibility was investigated and the percent of relative standard deviations (%RSDs) for 180 and 720 ng mL^{-1} of ARP (n=11) were 4.5% and 5.2%, respectively. The minimum sampling rate calculated about 70 samples per hour.

Influence of interfering substances

In order to validate of the possible analytical application of the method, interference effect of some common ions, excipients in pharmaceutical preparations and some amino acids were studied by recovering 180 ng mL^{-1} (4.0×10^{-7} mol L^{-1}) of ARP in presence of each substance. The tolerance of each substance was taken as the largest amount yielding an error of less than 3σ in the analytical signal of 180 ng mL^{-1} ARP (σ is the standard deviation in the response obtained from 11 times determination of 180 ng mL^{-1} of ARP). 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. The results have been shown in Table 1.

Table 1 Effect of foreign substances for the determination of 180 ng mL^{-1} ARP

Substance	Concentration Ratio of Substance to ARP
Lactose, Valine, Leucine, Urea, Serine, Threonine, Sucrose, Glucose, Fructose, Saccharin, Starch, K^+, Cl^-, PO_4^{3-}, Alanine	1000
Glycine, Proline, Na^+, HCO_3^-, CO_3^{2-}, Cystine, Tyrosine, Fe^{2+}, Mg^{2+}, Oxidized glutathione	100
Aspartic acid, Tryptophan, Glutathione	25
Ascorbic acid, Cysteine	5

Application

In order to evaluate the applicability of the proposed method, ARP tablets were analyzed to determine their ARP contents. Also recovery of ARP from human plasma was investigated. The results are shown in Table 2. The obtained results from analyzing of tablet samples were also certified by a spectrophotometric method provided for analyzing ARP in tablets [33]. The method was a UV spectrometric method in 219 nm using methanol as solvent. Statistical analysis of the results using student t-test and the variance ratio F-test showed no significant difference between the performance of two methods as regards to accuracy and precision. The results are presented in Table 3.

Table 2 Results for the determination of ARP in tablets and human plasma

Real Sample	Sample No.	Added (μg mL^{-1})	Found (μg mL^{-1})[d]	Recovery of Added %
Tablet (Abilizol[a])	1	2.00	2.00±0.14	100.0
	2	4.00	3.96±0.27	99.0
	3	8.00	7.89±0.47	98.6
Tablet (Biopiprazole[b])	1	2.00	2.02±0.08	101.0
	2	4.00	4.11±0.18	102.8
	3	8.00	7.91±0.35	98.9
Tablet (Abilify[c])	1	2.00	2.08±0.11	104.0
	2	4.00	3.86±0.35	96.5
	3	8.00	8.17±0.09	102.1
Plasma	1	0.100	0.092±0.030	92.0
	2	0.500	0.493±0.032	98.6
	3	1.500	1.567±0.061	104.4

[a] Abilizol 5 mg tablet, Sobhan Darou Co., Iran.

[b] Biopiprazole 15 mg tablet, Bakhtar Bioshimi Co., Iran.

[c] Abilify 10 mg tablet Bristol Myers Squibb, Turkey.

[d] Mean values of four replications

Table 3 Analysis of two formulations containing ARP using the proposed method and the reference method

Sample	Nominal value	Analytical Results[a]		t-test[c]	F-test[d]
		Proposed method	Ref. method[b]		
ARP Tablet	5 mg per tablet	5.24±0.14	5.09±0.05	1.42	7.84
ARP Tablet	10 mg per tablet	10.38±0.21	10.19±0.07	2.42	9.0

[a] Mean values of four replications.

[b] a UV spectrometric method in 219 nm using methanol as solvent.

[c] Student t-test calculated, theoretical value=3.182 (P=0.05).

[d] F-test calculated, theoretical value=9.28 (P=0.05).

Response characteristics

In Table 4 some response characteristics of the proposed method are compared with recently reported methods. As is quite obvious from Table 4, only mass spectrometry is more sensitive than the proposed method. All other methods have a narrower linear dynamic range, lower throughput and are less sensitive than the proposed method. Although, analytical techniques coupled with a separation method [7-9, 13, 15, 19-21, 28, 29], besides chemical information for ARP, provide multi analyte information about related species, compounds and metabolites presented in the sample. However, each of these methods often offers its own set of advantages and disadvantages. There are some disadvantages, such as several time-consuming manipulations, special training, or requirement of comparatively expensive equipment and they are not readily amenable to be cost-effective or to miniaturize instrumentation. The proposed method is a simple and fast analytical tool for obtaining of preliminary chemical information about ARP, prior the use of more complex instrumental techniques. In addition, the proposed CL method has been successfully used to determine ARP in pharmaceutical and plasma samples.

Table 4 Response characteristics of the proposed method in comparison with other recently methods for the determination of ARP

Method	Technique	LOD (ng mL^{-1})	LDR (ng mL^{-1})	Speed (h^{-1})	Ref.
Chromatography	HPLC-UV	5.0	(20-200)×10^3	4	13
	HPLC-UV	16	50-2500	2	8
	Ion pair RPLC[a]	5.0	50-2500	2	8
	HPLC	50	(40-160)×10^3	6	15
	UPLC[b]	10	(40-160)×10^3	20	15
	HPLC-UV	2850	(10-60)×10^3	NR[i]	7
	RP-HPLC	411	(20-60)×10^3	7	9
Mass Spectrometry	LC-MS/MS	NR	0.10-100	25	19
	SPE[c]-UPLC-MS/MS	NR	0.05-80	50	20
	LC–ESI-MS[d]	NR	0.20-60.01	16	21
Spectrophotometry	UV-Vis	220	(5-30)×10^3	NR	36
	UV-Vis	300	(10-120)×10^3	NR	31
	UV-Vis	NR	(10-60)×10^3	NR	32
Electrochemistry	LSV[e]	50	(0.1-5)×10^3	NR	37
	AdSV[f]	1.0	(4-40)	NR	37
	AdSV	50	(5-70)×10^3	NR	38
Electrophoresis	CE[g]	1.5	0.5-50	30	29
	CE	2.0	5.0-100	5	28
Luminescence	CL[h]	0.9	1.8-18.0 18-35900	70	PM[j]

[a] Reversed-phase liquid chromatography.

[b] Ultra-performance liquid chromatography.

[c] Solid phase extraction.

[d] Liquid chromatography-electrospray ionization-mass spectrometry.

[e] Linear scan voltammetry.

[f] Adsorptive stripping voltammetry.

[g] Capillary electrophoresis.

[h] Chemiluminescence.

[i] Not reported.

[j] Present method.

Detailed CL mechanism

Solution of $Ru(phen)_3^{2+}$ is orange and its color changes to green immediate after mixing with oxidizing agent, Ce(IV) solution, and production of $Ru(phen)_3^{3+}$ [67, 68]. During about 3 minutes after mixing of $Ru(phen)_3^{2+}$ with Ce(IV), the color of the mixture changes slowly from green to orange. UV-Vis spectrum of Ce(IV) solution (spectrum a), ARP (spectrum b), $Ru(phen)_3^{2+}$ (spectrum g) and the mixture of $Ru(phen)_3^{2+}$-Ce(IV) with one minute interval times (spectrum c to f) are shown In fig. 7.

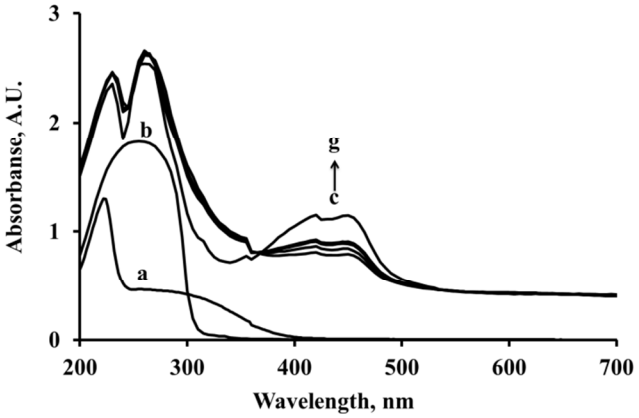

Fig. 7 UV-Vis spectrum of a) Ce(IV) b) ARP c to f) mixture of $Ru(phen)_3^{2+}$-Ce(IV) with 1 minute intervals g) $Ru(phen)_3^{2+}$. Conditions: a) 2 mL Ce(IV) (3.0×10^{-4} mol L^{-1} in 0.007 mol L^{-1} of H_2SO_4) b) ARP (45.0 µg mL^{-1}) c to f) 2 mL $Ru(phen)_3^{2+}$ (5.0×10^{-5} mol L^{-1}) and 1.0 mL Ce(IV) (3.0×10^{-4} mol L^{-1} in 0.007 mol L^{-1} of H_2SO_4) g) 2 mL $Ru(phen)_3^{2+}$ (5.0×10^{-5} mol L^{-1})

As can be seen in fig. 7, absorbance in the range 400-500 nm which is related to $Ru(phen)_3^{2+}$ complex decreases immediately after mixing the $Ru(phen)_3^{2+}$ solution with Ce(IV) solution (spectrum c) and it increases slowly to its equilibrium value after about 3 minutes (spectrum d to f). The reason is that, the resulting $Ru(phen)_3^{3+}$ produced in the reaction of $Ru(phen)_3^{2+}$ with acidic Ce(IV), is a powerful oxidant and oxidizes water into O_2 and protons [69]. Therefore, it returns slowly to its reduced state. If there was a reducing agent in the reaction media, it can reduce $Ru(phen)_3^{3+}$ very fast. The electrons from reducing agent transfer to the π*-orbital of phenanthroline ligand and the $Ru(phen)_3^{2+}$ π* metal-to-ligand charge transfer (MLCT) excited state can be produced [70]. The excited electron then undergoes intersystem crossing to the lowest triplet state of $Ru(phen)_3^{2+}$, from where emission occurs [71].

In order to confirm the mechanism proposed above, some CL pathways might be investigated for the $Ru(phen)_3^{2+}$-Ce(IV)-ARP CL system, involving the formation of Ce(III)*, oxidation products in excited state and $[Ru(phen)_3^{2+}]$*.

To explore the possible CL mechanism, some experiments performed and following results were obtained.

1 A weak CL intensity was observed when $Ru(phen)_3^{2+}$ solution was mixed with acidic Ce(IV) solution. Enhancement in CL intensity was detected when ARP solution was present in the mixture of $Ru(phen)_3^{2+}$ and acidic Ce(IV).

2 Fluorescence spectrum of ARP (λ_{ex} = 255 nm) was scanned using spectrofluorometer (Jasco, model FP-750) using batch mode. Fluorescence of ARP (λ_{max} = 440 nm) was disappeared when Ce(IV) solution was added into the cuvette and new peak was appeared at 370 nm. These are due to oxidation of ARP and formation of Ce(III) that is a well-known fluorescent ion [72].

3 Fluorescence emission spectrum of $Ru(phen)_3^{2+}$ (λ_{ex} = 450 nm), had a maximum at 580 nm.

4 CL spectra of mixtures including Ce(IV)-ARP (Fig. 8a), Ce(IV)-$Ru(phen)_3^{2+}$ (Fig. 8b) and Ce(IV)-$Ru(phen)_3^{2+}$-ARP (Fig. 8c) were obtained using spectrofluorometer (Jasco, model FP-750). No detectable CL intensity 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 same maximum emission wavelength at 580 nm which is same as maximum fluorescence emission of $Ru(phen)_3^{2+}$. This indicates that the CL spectra are independent of ARP and the emitter is $[Ru(phen)_3^{2+}]$*.

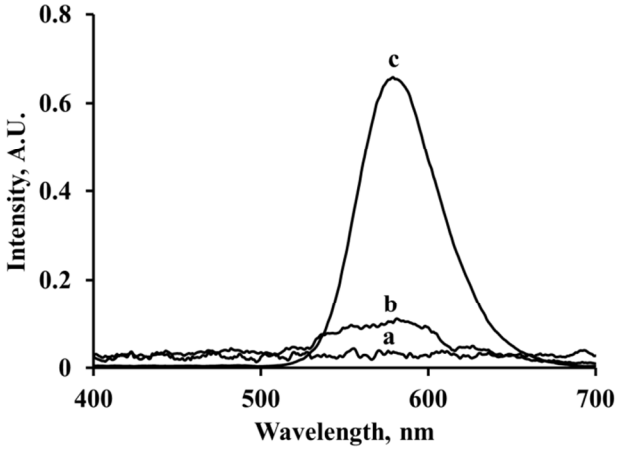

Fig. 8 CL spectrum of a) Ce(IV)-ARP, b) Ce(IV)-$Ru(phen)_3^{2+}$, c) Ce(IV)-$Ru(phen)_3^{2+}$-ARP (because of high intensity, the response of spectrum c was divided by 10). Conditions: a) 2 mL ARP (1.0 µg mL^{-1}) and 400 µL Ce(IV) (3.0×10^{-3} mol L^{-1} in 0.07 mol L^{-1} of H_2SO_4), b) 2 mL $Ru(phen)_3^{2+}$ (2.0×10^{-3} mol L^{-1}) and 400 µL Ce(IV) (3.0×10^{-3} mol L^{-1} in 0.07 mol L^{-1} of H_2SO_4), c) 2 mL $Ru(phen)_3^{2+}$ (2.0×10^{-3} mol L^{-1}), 200 µL ARP (1.0 µg mL^{-1}) and 400 µL Ce(IV) (3.0×10^{-3}mol L^{-1} in 0.07 mol L^{-1} of H_2SO_4)

ARP is a tertiary amine and from previous studies, the oxidation of tertiary amines is understood to produce a short-lived radical cation. The α-carbon is then deprotonated, yielding a strongly reducing intermediate. This reduces the $Ru(phen)_3^{3+}$ (produced by oxidant) to the excited state that subsequently emits light [66, 73-75]. The changes in the concentration of $Ru(phen)_3^{2+}$ was also obtained at 450 nm after mixing with Ce(IV) solution. In this way, time course curve for $Ru(phen)_3^{2+}$ was obtained in presence (Fig. 9b) and in absence (Fig. 9a) of ARP as reducing agent. Because there is no interference from Ce(IV), ARP and Ce(III) at 450 nm, the absorbance at 450 nm is proportional to $Ru(phen)_3^{2+}$ concentration. As can be seen in Fig. 9, for solution which was include ARP (Fig. 9b), smaller decrease in absorbance and faster equilibrium occurred. This phenomenon shows that ARP can reduce $Ru(phen)_3^{3+}$ and it can speedup production of $Ru(phen)_3^{2+}$ from $Ru(phen)_3^{3+}$. After injection of Ce(IV) solution and in presence of ARP, $Ru(phen)_3^{2+}$ received to highest concentration after about 50 seconds but in absence of ARP it lasts about 75 seconds.

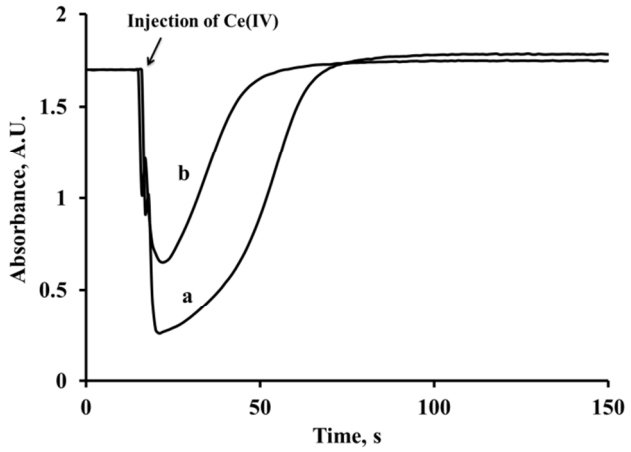

Fig. 9 Time course curve of $Ru(phen)_3^{2+}$ at 450 nm after mixing with Ce(IV) solution, a) in absence and b) in presence of ARP. Conditions: 2 mL $Ru(phen)_3^{2+}$ (1.0×10^{-3} mol L^{-1}), 1.0 mL Ce(IV) (3.0×10^{-3} mol L^{-1} in 0.07 mol L^{-1} of H_2SO_4), 100 µL ARP (45.0 µg mL^{-1}) only in time course b

According to the above discussion, the following mechanism is proposing for the CL reaction of ARP.

$Ru(phen)_3^{2+} + Ce(IV) \rightarrow Ce(III) + Ru(phen)_3^{3+}$

$ARP + Ce(IV) \rightarrow Ce(III) + ARP^{+\bullet}$

$ARP^{+\bullet} \rightarrow ARP^{\bullet} + H^{+}$

$Ru(phen)_3^{3+} + ARP^{\bullet} + H_2O \rightarrow [Ru(phen)_3^{2+}]^* + \text{ARP fragments}$

$[Ru(phen)_3^{2+}]^* \rightarrow Ru(phen)_3^{2+} + h\upsilon$

Conclusions

A new method based on the CL of $Ru(phen)_3^{2+}$ and acidic Ce(IV), was proposed for the quantification of ARP. The method is simple, rapid and sensitive for the determination of ARP in pharmaceuticals and human plasma. Some common sugars, amino acids and ions hadn't significant interference effect in the quantification of ARP indicating high accuracy and suitability for determining of ARP in human fluids and quality assurance in drug formulations. One future trend might be the combination of the proposed CL system with liquid chromatography equipment, developing a proper technique for the determination of ARP in various complex matrixes and pharmaceuticals.

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Notes and references

Department of Science, Golestan University, Gorgan, I.R Iran. Tel and Fax: 1736236490; E-mail: a.mokhtari@gu.ac.ir.

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