



Anal. Bioanal. Chem. Res., Vol. 2, No. 1, 52-59, June 2015.

Terbium Sensitized Chemiluminescence Method for the Determination of Rabeprazole -Application to Pharmaceutical Analysis and Dissolution Studies

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(Received 20 August 2014, Accepted 29 April 2015)

A simple and sensitive chemiluminescence-based method was established for the determination of rabeprazole. The proposed method was based on the enhancing effect of rabeprazole on Ce(IV)-Na₂SO₃-Tb(III) chemiluminescence reaction. A possible mechanism was discussed for chemiluminescence system by studying UV-Vis, fluorescence and chemiluminescence spectra. The effects of various chemical parameters were investigated and optimized. Under the optimum conditions, the enhanced chemiluminescence intensity was directly proportional to the concentration of rabeprazole in the range of 0.015-0.2 μg ml⁻¹, with a detection limit of 6 ng ml⁻¹. The proposed method was applied to the analysis of pharmaceutical formulations and human plasma samples and to the dissolution study of rabeprazole tablets with satisfactory results. The results indicated that more than 95% of the labeled amount of rabeprazole was dissolved over 30 min in the basic medium, while only 10% of rabeprazole was released in acidic medium.

Keywords: Sensitized chemiluminescence, Terbium(III), Cerium(IV)-Sulfite, Rabeprazole, Dissolution study, Human plasma

INTRODUCTION

Proton pump inhibitors (PPIs) are the most important drugs in treating acid-related diseases. PPIs inhibit selectively and irreversibly the gastric H⁺/K⁺-ATPase (the proton pump) that accomplishes the final step in acid secretion. All PPIs inhibit both basal and stimulated secretion of gastric acid, independent of the nature of parietal cell stimulation. Rabeprazole (2-[[[4-(3-Methoxypropoxy)-3-methyl-2-pyridinyl] methyl] sulfinyl]-1 H-benzimidazole Fig. 1) is a substituted benzimidazole proton pump inhibitor. It is effective in healing duodenal ulcer, gastric ulcers, and erosive oesphagitis, and when co-prescribed with antibiotics, in eradicating H. pylori infection. Rabeprazole undergoes activation over a greater pH range than other proton pump inhibitors, and converts to sulphenamide form more rapidly than other PPIs. More rapid activation probably accounts for rabeprazole faster inhibition of H⁺/K⁺-ATPase activity and acid transport [1,2].

Biological activity of a drug can be depended on the

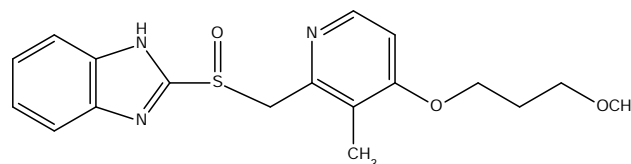


Fig. 1. Chemical structure of rabeprazole.

release rate of drug in the body after administration. Thus, the *in vitro* dissolution may relevant for the prediction of the *in vivo* performance of a drug product. Based on this general consideration, *in vitro* dissolution tests for immediate release solid oral dosage forms, such as tablets and capsules, are used to (1) assess the lot-to-lot quality of a drug product; (2) guide development of new formulations; and (3) ensure continuing product quality and performance after certain changes [3]. Therefore, the determination of dissolution profiles for active compounds in pharmaceutical formulations is required for research and quality control laboratories.

Although there are several analytical methods including spectrophotometry [4,5], high-performance liquid

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chromatography [6-11], high-performance liquid chromatography with solid-phase extraction [12], liquid chromatography-mass spectrometry [13,14] for the determination of rabeprazole in pharmaceutical formulations and human plasma, a few analytical methods have been reported for dissolution study of rabeprazole tablets in the literature [15].

Chemiluminescence (CL)-based analytical methods have extensively been applied for determination of pharmaceutical and biological compounds due to some advantages such as simplicity, low detection limit, large calibration ranges and short analysis times. CL in aqueous phase systems is usually generated by redox reactions in which, electronically-excited molecules are produced and subsequently deactivate with photon emission. CL reactions have been classified as direct and indirect or sensitized CL. Indirect or sensitized CL is based on energy transfer from the excited species to a fluorophore, which then produces light emission [16]. Recently, lanthanide ions have been applied in the CL analyses as sensitizer for determination of several compounds [17-21].

The oxidation of sulfite by Ce(IV) is known to produce a weak CL signal [22]. Enhancing the CL intensity has become critical in order to increase the sensitivity and expand the range of applications. This weak CL signal can be enhanced by fluorescent [23] and non-fluorescent enhancers [24,25]. There are also several reports indicating that the CL signal of Ce(IV) and sulfite reaction can be greatly increased by lanthanide ions [26-30].

In the present study, we showed that the weak CL signal from cerium(IV)-sulfite system is greatly enhanced in the presence of Tb^{3+} and rabeprazole. Based on this phenomenon, a simple and sensitive CL method was developed for determination of low concentrations of rabeprazole. The method possesses a good accuracy and precision and was applied to the determination of rabeprazole in pharmaceutical formulations and human plasma samples and to the dissolution study of rabeprazole tablets with satisfactory results.

EXPERIMENTAL

Apparatus

The chemiluminescence signals were monitored by

LUMAT LB 9507 chemiluminometer (Berthold; www.berthold.com). UV-Vis spectra were recorded on a Cary-100 Spectrophotometer (Varian; www.varianinc.com). CL spectra were recorded with RF-540 spectrofluorimeter (Shimadzu, Japan) using flow mode with the excitation light source being turned off. The fluorescence spectra were also recorded by the same instrument under normal conditions. Continuous flow system which used for recording CL spectra consisted of a ismatec peristaltic pump, PTFE tubing (0.8 mm i.d.), and the T shape flow cell made by glass tube (1 mm i.d. and 2 mm o.d.) which located directly facing the window of the photomultiplier tube.

Reagents

All reagents used were of analytical reagent grade. Double-distilled water (obtained from Ghazi Serum Co. Tabriz, Iran) was used throughout the experiment. Pure rabeprazole sodium was obtained from Aburaihan Pharm. Co (Tehran, Iran). A $200 \mu\text{g ml}^{-1}$ stock standard solution of rabeprazole sodium was prepared daily by dissolving 20.0 mg of drug in deionized water and diluting to the mark in a 10 ml volumetric flask. Dilute standard solutions were prepared just before use. Two brands of rabeprazole tablets were purchased from a local drugstore. $\text{Ce}(\text{SO}_4)_2 \cdot 4\text{H}_2\text{O}$, Na_2SO_3 , H_2SO_4 , HCl , NaOH , ZnSO_4 , $\text{Ba}(\text{OH})_2$, and $\text{Na}_2\text{B}_4\text{O}_7$ were obtained from Merck (Darmstadt, Germany) and $\text{TbCl}_3 \cdot 6\text{H}_2\text{O}$ was obtain from Acros Organics (Geel, Belgium). Ce(IV) solution (0.005 M) was prepared in 0.05 M H_2SO_4 . A 0.01 M Na_2SO_3 was prepared daily.

General Procedure

Chemiluminescence analyses were carried out in a 3 ml tube, in the batch condition. Briefly, 50 μl of Na_2SO_3 (0.01 M) and 65 μl of $\text{TbCl}_3 \cdot 6\text{H}_2\text{O}$ (0.01 M) were added into the cell. Then an appropriate volume of sample or standard rabeprazole solution was added and the final volume was reached to 1.0 mL with distilled water. After injection of 100 μl of Ce(IV) (0.005 M in 0.05 M H_2SO_4) by an automatic injector, monitoring of CL signal vs. time was started automatically. Maximum CL intensity was used as analytical signal.

Preparation Procedure for Tablets

Five rabeprazole tablets were weighed to find the

average mass of each tablet and then powdered and mixed. An accurately weighed portion of homogenized powder containing about 2 mg rabeprazole was dissolved in about 10 ml deionized water. The solution was filtered into a 100 ml volumetric flask and the residue was washed several times with water, and then diluted to the mark. An appropriate portion of this sample solution was taken for determination of rabeprazole according to the general procedure.

Dissolution Study Conditions

The dissolution study was performed according to the literature [15]. The study was conducted in two steps, both using paddle, at stirring speed of 75 rpm and temperature of 37 ± 0.5 °C.

Acidic step. Each tablet, containing ~20 mg of rabeprazole, was placed into 900 ml of 0.1 M hydrochloric acid for 2 h, then tablets were removed and the amount of rabeprazole determined by the proposed method.

Basic step. After 2 h in the acidic medium, the new set of tablets was added into the borate buffer pH 9.0 dissolution medium (900 ml). Aliquots of 3 ml were withdrawn of each vessel at 5, 10, 15, 30, 45 and 60 min and equal volume of fresh medium was replaced to maintain a constant total volume. Samples were diluted with water to a suitable concentration and assayed by proposed method.

Preparation Procedure for Human Plasma Samples

Human plasma samples were obtained from Blood Transfusion Center (Tabriz, Iran). A 500 μ l aliquot of plasma was placed into a centrifuge tube and spiked by adding appropriate volumes of rabeprazole standard solution ($10 \mu\text{g ml}^{-1}$). 2.0 ml of 0.1 M $\text{Ba}(\text{OH})_2$ and 1.8 ml of 0.1 M ZnSO_4 were added into the tube for precipitation of proteins [31]. Then the solution was centrifuged for 15 min. The supernatant solution was transferred into a 5.0 ml volumetric flask and diluted to the mark with water. Appropriate portion of this solution was analyzed according to the general procedure.

RESULTS AND DISCUSSION

A series of preliminary experiments with several CL systems including $\text{Ce}(\text{IV})\text{-Na}_2\text{SO}_3$, $\text{Ce}(\text{IV})\text{-Na}_2\text{S}_2\text{O}_3$.

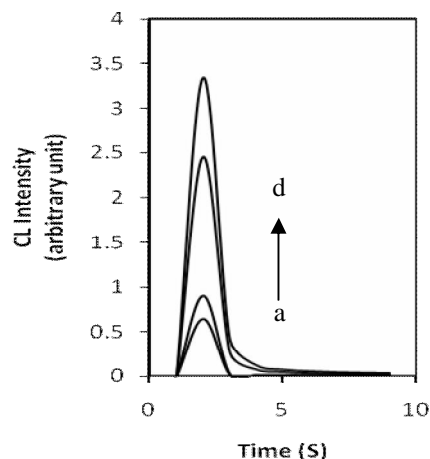


Fig. 2. Kinetic curve for $\text{Ce}(\text{IV})\text{-Na}_2\text{SO}_3$ CL system, (a) alone, (b) in the presence of rabeprazol, (c) in the presence of Tb^{3+} , (d) in the presence of Tb^{3+} and rabeprazol. Conditions: $\text{Ce}(\text{VI})$, 5×10^{-4} M; Na_2SO_3 , 5×10^{-4} M; H_2SO_4 , 5×10^{-3} M; Tb^{3+} 6.5×10^{-4} M and rabeprazol, $0.05 \mu\text{g ml}^{-1}$.

$\text{KMnO}_4\text{-Na}_2\text{SO}_3$ and $\text{KMnO}_4\text{-Na}_2\text{S}_2\text{O}_3$ in the absence and presence of $\text{Tb}(\text{III})$ and $\text{Eu}(\text{III})$ ions was performed for rabeprazole determination. The best results were obtained by $\text{Ce}(\text{IV})\text{-Na}_2\text{SO}_3\text{-Tb}(\text{III})$ system. As shown in Fig. 2a the redox reaction of $\text{Ce}(\text{IV})\text{-Na}_2\text{SO}_3$ produces a weak CL signal in the acidic medium, which upon addition of Tb^{3+} , is enhanced (Fig. 2, curve c). When rabeprazole is added into the $\text{Ce}(\text{IV})\text{-Na}_2\text{SO}_3\text{-Tb}(\text{III})$ system, a notable enhancement in the CL intensity is observed (Fig. 2, curve d). However, the addition of rabeprazole without Tb^{3+} has only little effect on the CL intensity (Fig. 2 curve b).

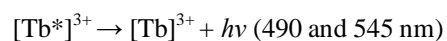
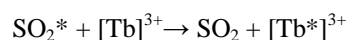
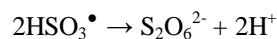
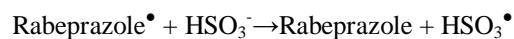
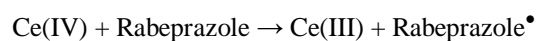
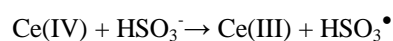
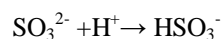
Possible Mechanism for the CL Enhancement and Effect of Rabeprazole

It has been proposed that the emitting species for $\text{Ce}(\text{IV})\text{-Na}_2\text{SO}_3$ CL reaction is excited sulfur dioxide, which emits weak light at wavelengths longer than 300 nm [32,33]. As mentioned before, when a lanthanide ion such as $\text{Tb}(\text{III})$ is present in the system, the CL intensity fairly enhanced. This enhancement indicates that energy transfer process occurs from the excited SO_2^* to $\text{Tb}(\text{III})$ ions. Furthermore, when $\text{Tb}(\text{III})$ and rabeprazole are added simultaneously into the $\text{Na}_2\text{SO}_3\text{-Ce}(\text{IV})$ system, a greater

enhancement in the CL intensity is observed. It can, therefore, be concluded that more SO_2^* is generated in the presence of rabeprazole because it is oxidized by cerium(IV) to form an intermediate radical, which reacts with sulfite and initiates a free radical reaction.

In order to confirm the proposed CL mechanism, we recorded the CL and UV spectra for our system. As shown in Fig. 3, the absorbance intensity of acidic cerium(IV)- Na_2SO_3 solution increases after mixing with rabeprazole (Fig. 3). The maximum of UV spectrum at 253 nm is the characteristic UV spectrum of cerium(III) [34]. This indicates that rabeprazole is oxidized by acidic cerium(IV).

The chemiluminescence spectra of Na_2SO_3 -Ce(IV)- Tb^{3+} and Na_2SO_3 -Ce(IV)- Tb^{3+} -rabeprazole systems are shown in Fig. 4. The emission peaks of both systems are located at around 490 and 545 nm which is the characteristic fluorescence spectra of terbium. This clearly indicates that the emitting species is excited $\text{Tb}(\text{III})$, and there must be energy transfers in the reaction system. Based on these considerations, a sensitized CL mechanism can be proposed as follows [35]:



Optimization of Chemical Conditions

To obtain the parameters which gave the greatest chemiluminescence signal with rabeprazole, the effects of several analytical variables such as the concentration of Na_2SO_3 , Ce(IV), H_2SO_4 and Tb^{3+} on the CL intensity were investigated.

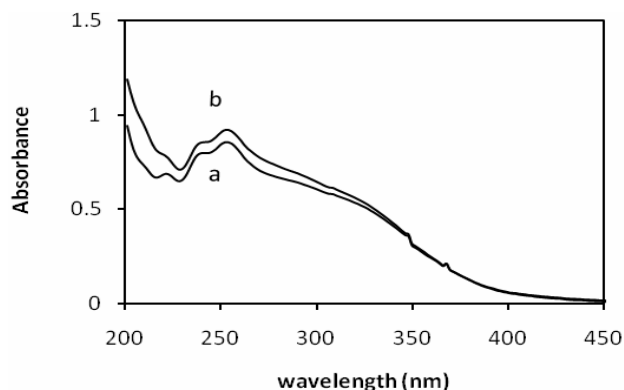


Fig. 3. Absorbance spectrum of Ce(IV)- Na_2SO_3 system (a) in the absence and (b) in the presence of rabeprazole, Ce(IV), 5×10^{-4} M, H_2SO_4 , 5×10^{-3} M and Na_2SO_3 , 5×10^{-4} M; rabeprazole, $2 \mu\text{g ml}^{-1}$.

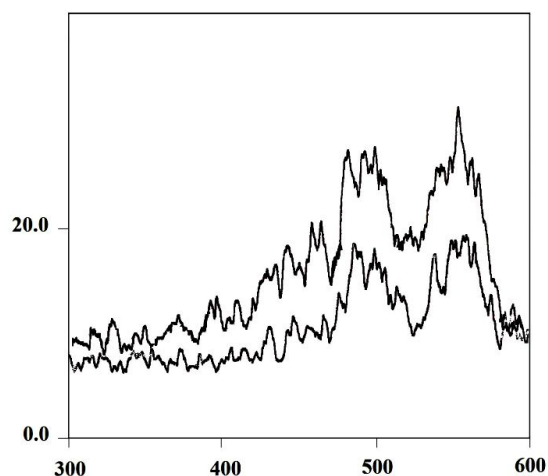


Fig. 4. CL spectrum of Ce(IV)- Na_2SO_3 - Tb^{3+} system (lower spectrum) in the absence and (upper spectrum) in the presence of rabeprazole obtained with continuous flow of reagents: Ce(IV), 1.0×10^{-3} M and H_2SO_4 , 0.01 M in one line and Na_2SO_3 , 1.0×10^{-3} M and 1.0×10^{-3} M with or without rabeprazole ($0.2 \mu\text{g ml}^{-1}$) in other line.

The effect of Na_2SO_3 concentration on the CL intensity over the range 10^{-4} - 1.5×10^{-3} M was investigated. According to the results (Fig. 5a) the CL intensity was increased as the Na_2SO_3 concentration increased with maximum CL intensity at 5×10^{-4} M. Above Na_2SO_3

concentration, however, the CL intensity declined; therefore this amount was used as optimum concentration.

In order to examine the effect of Ce(IV) concentration, solutions with different concentration of $\text{Ce}(\text{SO}_4)_2 \cdot 4\text{H}_2\text{O}$ were prepared over the range of 2×10^{-4} to 7.5×10^{-3} M. It was observed that the maximum CL response was achieved for the 5×10^{-4} M Ce(IV) (Fig. 5b). At lower Ce(IV) concentrations the number of excited intermediates is decreased and the response is diminished.

The influence of sulfuric acid concentration in Ce(IV) solution on the CL signal was also studied. As shown in Fig. 5c, the CL signal increased until 5×10^{-3} M and then decreased in high concentrations. The decrease in CL intensity at low concentrations is probably due to the hydrolysis of Ce(IV) forming cerium hydroxide. Therefore, 0.005 M sulfuric acid was used for further work.

The effect of Tb^{3+} concentration in the range of 10^{-4} to 10^{-3} M on the CL intensity was also examined (Fig. 5d). The CL intensity is increased by increasing the Tb^{3+} concentration up to 6.5×10^{-4} M and then decreased in high concentrations. Because the generated energy of Na_2SO_3 -Ce(IV) CL reaction was limited, just a certain amount of Tb^{3+} could be excited by the energy transfer process.

Analytical Application of the CL System

Under the optimum conditions described above, the analytical figures of merit for the determination of rabeprazole was investigated. The CL response was found to be linear in the concentration range of 0.015 - $0.2 \mu\text{g ml}^{-1}$ with a limit of detection (3s) of 6 ng ml^{-1} . The regression equation was $\Delta I = (14.43 \pm 0.33) C + (0.03 \pm 0.03)$, $R^2 = 0.9974$, where $\Delta I = I - I_0$ is the difference between CL intensity in the presence of rabeprazole (I) and in its absence (I_0), and C is concentration of rabeprazole in $\mu\text{g ml}^{-1}$. The relative standard deviation (RSD) was obtained to be 0.14, 2.80 and 2.87% for five determinations of 0.05, 0.075 and $0.12 \mu\text{g ml}^{-1}$ rabeprazole, respectively. The results indicate that this CL system has good linearity, relatively high sensitivity and suitable precision. Comparison between the proposed method and some other reported analytical methods for the rabeprazole quantification is shown in Table 1. As can be seen, the developed method has better limit of detection than most of other methods. Moreover it is very simple and inexpensive.

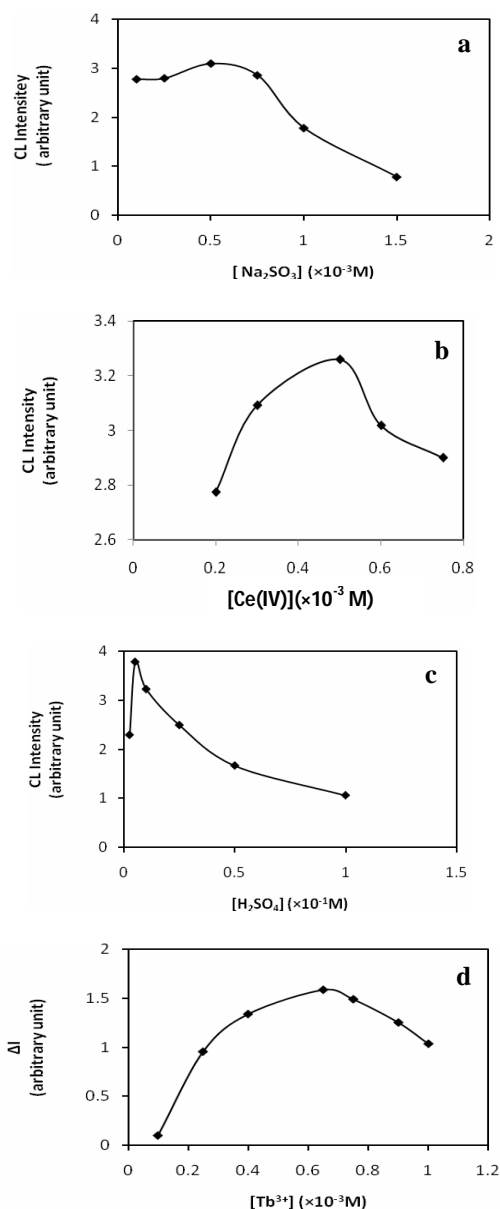


Fig. 5. Optimization of the CL reaction conditions: (a) Effect of Na_2SO_3 concentration. Conditions: Ce(IV), 6×10^{-4} M, H_2SO_4 , 6×10^{-3} M, Tb^{3+} , 5×10^{-4} M, rabeprazole $0.1 \mu\text{g ml}^{-1}$ (b) Effect of Ce(IV) concentration. Conditions: Na_2SO_3 , 5×10^{-4} M; other conditions are as in a; (c) Effect of H_2SO_4 concentration. Conditions: Ce(IV), 5×10^{-4} M; other conditions are as in b (d) Effect of Tb^{3+} concentration. Conditions: H_2SO_4 , 5×10^{-3} M, other conditions are as in c.

Table 1. Comparison of the Developed CL Method for the Determination Rabeprazole with some Previously Published Methods

Method	LOD (ng ml ⁻¹)	Linear range (µg ml ⁻¹)	Ref.
Spectrophotometric method	550	4-44	[4]
LC	20	0.5-50	[6]
LC	65.65	0.2-2	[9]
SPE-LC	20 ^a	0.02-1	[11]
LC-MS	2 ^a	0.002-0.8	[12]
LC-MS-MS	0.00014 ^a	0.00014-0.0096	[13]
CL	6	0.015-0.2	This work

^aLOQ.**Table 2.** Results for Determination of Rabeprazole in Pharmaceutical Samples (Tablets)

Sample	Label (mg/capsule)	(Recovery + RSD)(%)	t-statistic ^a
Brand A	20	106 ± 4.7	2.6
Brand B	20	102 ± 3.4	1.9

^at-critical = 4.3 for n = 2 and P = 0.05.

Study of Interferences

In order to evaluate the selectivity of the proposed method, the effects of some common inorganic ions and organic compounds on the determination of 0.05 µg ml⁻¹ rabeprazole were investigated. The tolerable concentration ratios for interferences in relative error of <5% were over 2000 for Na⁺, K⁺, Zn²⁺, Mg²⁺, Cl⁻, NO₃⁻, sucrose, 1500 for Fe³⁺, 1000 for glycine, vitamin B₂, 750 for alanine, 700 for lactose, glucose, 400 for Cu²⁺, 250 for Ca²⁺, 200 for uric acid, 100 for L-cysteine, and 50 for pantaprazole, omeprazole, ascorbic acid and vitamin B₁. As can be seen, the amounts of most potentially interfering species in plasma are below their tolerable levels or can decrease with diluting, so there would be no interferences from these species in rabeprazole determination. These results demonstrate that the method possesses a good selectivity for the determination rabeprazole in pharmaceutical and biological samples.

Analytical Applications

Determination of rabeprazole in pharmaceutical samples. Rabeprazole was satisfactorily determined in two different brands of pharmaceutical formulations by using the proposed method. The results obtained and the labeled contents are given in Table 2. Statistical analysis of these

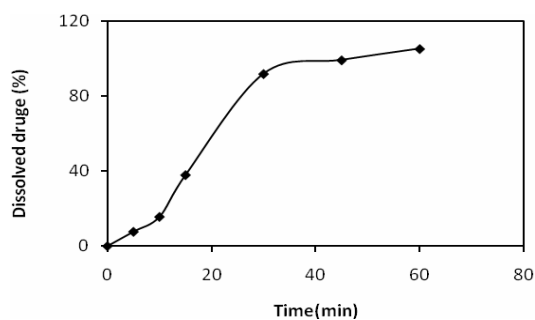
**Fig. 6.** Dissolved drug during the time in the dissolution studies of tablets of rabeprazol.

Table 3. Results for the Determination of Rabeprazol in Spiked Plasma Samples

Plasma Sample	Add ($\mu\text{g ml}^{-1}$)	Found ($\mu\text{g ml}^{-1}$) ^a	Recovery (%)	t-statistic
1	0.30	0.31 ± 0.01	102 ± 4.8	0.80
	0.60	0.60 ± 0.01	100 ± 2.3	0.04
	1.00	1.06 ± 0.04	105 ± 3.9	2.31
2	0.30	0.29 ± 0.006	98 ± 2.2	1.21
	0.60	0.60 ± 0.02	100 ± 3.0	0.28
	1.00	0.99 ± 0.03	99.6 ± 3.0	0.23

^aMean of three determinations \pm standard deviation. ^bt-critical = 4.3 for n = 2 and P = 0.05.

results using Student t-test showed that no significant differences between labeled and determined contents of rabeprazole are observed.

Dissolution studies. The proposed CL method was applied to the determination of the percentage of rabeprazole sodium remaining in the tablets after acidic step, and the result obtained was 97.2%. This indicated that less than 10% of the label amount was released in the acid step.

The tablets release profile obtained in the dissolution test, at the previously mentioned basic step conditions, showed an increase in rabeprazole concentrations in the dissolution medium with time (Fig. 6). It was also observed that more than 95% of the labeled amount of rabeprazole was dissolved over 30 min in the basic medium, being in accordance with the condition described earlier [15]. Therefore, the proposed method can be applied for dissolution study that is suitable for quality control of rabeprazole tablets.

Determination of rabeprazole in human plasma samples. The present method was easily applied to the determination of rabeprazol in spiked human plasma. A deproteinization step was found to be necessary. Also in order to avoid the matrix effects, the standard addition method was applied. The obtained results are shown in Table 3, which prove the applicability of the proposed method for bioanalytical samples.

CONCLUSIONS

A terbium sensitized chemiluminescence method was

developed for the determination of rabeprazole. Under the optimum condition, the CL intensity was proportional to the concentration of rabeprazole. The possible mechanism of the chemiluminescence is also discussed. The proposed CL method has good linearity, high sensitivity and good reproducibility, and can be applied to the determination of rabeprazole in pharmaceutical products and the dissolution study of rabeprazole tablets. It is suitable for rapid and accurate quality control of rabeprazole in tablet formulations.

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