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ARTICLES

A Sensitive Chemiluminescence Method for the Determination of Celecoxib in Pharmaceutical and Biological Samples[1]

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Abstract—A simple and sensitive chemiluminometric method was developed for the determination of celecoxib, a nonsteroidal anti-inflammatory drug. It is based on the sensitization of Ce(IV)–Na_2SO_3 chemiluminescence reaction by celecoxib in the presence of Tb(III) ions. The mechanism of chemiluminescence reaction was studied by obtaining fluorescence and chemiluminescence spectra. The effects of various chemical parameters on chemiluminescence intensity were investigated and optimized. Under the optimum conditions, a linear relationship was obtained between the enhanced chemiluminescence intensity and the concentration of celecoxib in the range of 0.01–0.15 μg/mL, with a limit of detection of 2.5 ng/mL. Study of interferences confirmed the selectivity of the developed method for analysis of pharmaceutical and biological samples. The method was applied to the determination of celecoxib in pharmaceutical formulations and human plasma samples, and also to dissolution studies with satisfactory results.

Keywords: celecoxib, chemiluminescence, cerium(IV), sodium sulfite, terbium(III)

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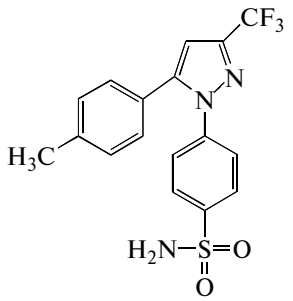
Celecoxib, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide is a nonsteroidal anti-inflammatory drug (**NSAID**), that is about 10–20 times more selective for COX-2 than for COX-1.



Chemical structure of celecoxib.

Celecoxib is as effective as other NSAIDs in the treatment of rheumatoid arthritis and osteoarthritis, and in trials it has caused fewer endoscopic ulcers than most other NSAIDs. Probably because it is a sulfonamide, celecoxib may cause rashes. It does not affect platelet aggregation at usual doses [1]. Investigations have revealed that celecoxib also has analgesic and anti-cancer properties. The chemopreventive effect of celecoxib on colon cancer [2] and its clinical effects on blood platelets [3] have been studied and reported. Furthermore, another study showed that celecoxib can reduce the polyps formation in patients with familial adenomatous polyposis [4]. It was reported that the maximum concentration of celecoxib in plasma was 0.797 mg/L after a single oral administration of 200 mg [5].

Several analytical methods have been reported for the determination of celecoxib in pharmaceutical formulations and human plasma. These methods include spectrophotometry [6, 7], fluorimetry [8, 9], adsorptive stripping voltammetry [10], high-performance liquid chromatography [11–14], liquid chromatography–mass spectrometry [15–17], solid-phase extraction–high-performance liquid chromatography [18] and micellar electrokinetic chromatography[19].

Chemiluminescence (**CL**)-based analytical methods show some advantages such as simplicity, rapidity and high sensitivity, which result in their extensive applications for the determination of pharmaceutical and biological compounds. In CL, reactions generally yield a reaction product in an electronic excited state which produces light on falling to the groundstate. CL reactions have been classified as direct and indirect or sensitized CL. Indirect or sensitized CL is based on a process of energy transfer from the excited species to a fluorophore, which then produces light emission [20].

There is a considerable interest in using the unique spectroscopic properties of some lanthanide ions, especially when they are chelated with appropriate organic ligands. The main advantages of lanthanide chelates in fluorescence spectrometry include large

[1] The article is published in the original.

Stokes shifts, narrow emission bands and long fluorescence lifetimes. These have led to numerous applications in various fields [21]. Recently, lanthanide ions have been applied in the CL analyses as sensitizers for the determination of pharmaceutical compounds [22–25]. The redox reaction between Ce(IV) and sulfite is known to produce a weak CL signal [26]. 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 [27] and non-fluorescent enhancers [28, 29]. There are also several reports indicating that the CL signal of Ce(IV) and sulphite reaction can be greatly increased by lanthanide ions [30–32].

In the present study, we showed that the weak CL signal from cerium(IV)–sulfite system is greatly enhanced in the presence of Tb(III) and celecoxib. Based on this phenomenon, a simple and sensitive CL method has been developed for the determination of low concentrations of celecoxib. The method possesses a good accuracy and precision and has been applied to the determination of celecoxib in pharmaceutical formulations and human plasma samples. To the best of our knowledge, no CL method has been reported for the determination of celecoxib.

EXPERIMENTAL

Apparatus. The chemiluminescence signals were monitored by LUMAT LB 9507 chemiluminometer (Berthold, Germany). 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.

Reagents. All reagents used were of analytical grade. Double-distilled water (obtained from Ghazi Serum Co., Tabriz, Iran) was used throughout the experiment. Pure celecoxib was obtained from Ipca Laboratories Limited (India). A 200 μg/mL stock standard solution of celecoxib was prepared by dissolving 20.0 mg of drug in methanol (Merck) and diluting to the mark in a 10 mL volumetric flask. Dilute standard solutions were prepared in water just before use. Three brands of celecoxib capsules were purchased from a local drugstore. $Ce(SO_4)_2 \cdot 4H_2O$, Na_2SO_3 and H_2SO_4 were obtained from Merck (Darmstadt, Germany) and $TbCl_3 \cdot 6H_2O$ was obtained from Acros Organics (Geel, Belgium). Ce(IV) solution (0.01 M) was prepared in 0.2 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 Na_2SO_3 (0.01 M) and 70 μL $TbCl_3 \cdot 6H_2O$ (0.01 M) were added into the cell. Then an appropriate volume of sample or standard celecoxib solution was added and the final volume was reached to 1.0 mL with distilled water. After injection of 80 μL Ce(IV) (0.01 M in 0.2 M H_2SO_4) by an automatic injector, monitoring of CL signal versus time was started automatically. Maximum CL intensity was used as analytical signal.

Preparation procedure for capsules. Five celecoxib capsules were weighed to find the average mass of each capsule and then powdered and mixed. An accurately weighed portion of homogenized powder containing about 2 mg celecoxib was dissolved in 4 mL methanol. The solution was filtered into a 10.0 mL volumetric flask, the residue was washed several times with methanol and then diluted to the mark with the same solvent. Working solution was prepared by appropriate dilution of this sample solution with water so that the final analytical concentration was within the working range.

Preparation procedure for human plasma samples. Human plasma samples were obtained from Blood Transfusion Center (Tabriz, Iran). A 1.0 mL aliquot of plasma was placed into a centrifuge tube and spiked by adding appropriate volumes of celecoxib standard solution (1.0 μg/mL); 1.0 mL of 10% trichloroacetic acid was added into the tube for precipitation of proteins. 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.

Dissolution study conditions. Dissolution studies were carried out according to Food and Drug Administration (USA) in 1000 mL of tribasic sodium phosphate (0.04 M, pH 12) containing 1% sodium lauryl sulfate, using II paddle method. A temperature of 37 ± 0.5°C was maintained in the system throughout the dissolution experiments. The rotation speed of the paddle was 50 rpm. Each capsule, containing 200 or 100 mg of celecoxib, was added into the dissolution medium. Aliquots of 3 mL were withdrawn of each vessel at 10, 20, 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 the proposed method.

RESULTS AND DISCUSSION

A series of preliminary experiments with several CL systems including Ce(IV)–Na_2SO_3, Ce(IV)–$Na_2S_2O_3$ $KMnO_4$–Na_2SO_3 and $KMnO_4$–$Na_2S_2O_3$ in the absence and presence of Tb(III) and Eu(III) ions was performed for celecoxib determination. The best results were obtained by Ce(IV)–Na_2SO_3–Tb(III) system. As shown in Fig. 1, curve *1*, the redox reaction of Ce(IV)–Na_2SO_3 produces a weak CL signal in the acidic medium, which is enhanced upon addition of Tb(III) (Fig. 1, curve *3*). When celecoxib was added into the Ce(IV)–Na_2SO_3–Tb(III) system, a notable enhancement in the CL intensity is observed (Fig. 1, curve *4*). However, the addition of celecoxib without

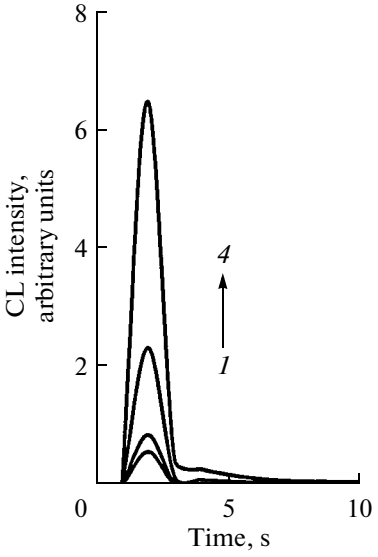


Fig. 1. Kinetic curve for Ce(IV)–Na_2SO_3 CL system: *1*—alone, *2*—in the presence of celecoxib, *3*—in the presence of Tb(III), *4*—in the presence of Tb(III) and celecoxib. Conditions: 8×10^{-4} M Ce(VI), 5×10^{-4} M Na_2SO_3, 0.016 M H_2SO_4, 7×10^{-4} M Tb(III) and 0.05 μg/mL celecoxib.

Tb(III) has only little effect on the CL intensity (Fig. 1, curve *2*).

Possible mechanism for the CL enhancement and effect of celecoxib. It has been proposed that the emitting species for Ce(IV)–Na_2SO_3 CL reaction is excited sulfur dioxide, which emits weak light at wavelengths longer than 300 nm [33, 34]. As mentioned before, when a lanthanide ion such as Tb(III) is present in the system, the CL intensity is fairly enhanced. This enchantment indicates that energy transfer occurs from the excited SO_2^* to Tb(III) ions. Furthermore, when Tb(III) and celecoxib are added simultaneously into the Na_2SO_3–Ce(IV) system, a much greater enhancement in the CL intensity is observed. This fact can be attributed to the more efficient intermolecular energy transfer from SO_2^* to celecoxib in the chelate (Tb–celecoxib) as a result of greater spectral overlap between emission profile of the donor and absorption spectrum of the acceptor. This follows by an intramolecular energy transfer from celecoxib to Tb(III).

In order to confirm the proposed CL mechanism, we recorded the CL and fluorescence emission spectra for our system. As shown in Fig. 2, the fluorescence emission of celeoxib shows a broad peak around 430 nm. In the presence of Tb(III), the intensity of celecoxib emission band decreases greatly, and the narrow emission bands of the Tb(III) appear at 490 and 545 nm, corresponding to $^5D_4 \longrightarrow {}^7F_6$ and $^5D_4 \longrightarrow {}^7F_5$ transitions of Tb(III), respectively [21]. This indicates that the Tb(III)–celecoxib complex has been formed and the intramolecular energy transfer has occurred between celecoxib and Tb^{3+}. The chemiluminescence spectra of Na_2SO_3–Ce(IV)–celecoxib, Na_2SO_3–Ce(IV)–Tb(III) and Na_2SO_3–Ce(IV)–Tb(III)–celecoxib systems are shown in Fig. 3. For the first system, a weak broad emission peak with maximum at around 500 nm was obtained, which belongs to SO_2^* [33]. It should be mentioned that for Na_2SO_3–Ce(IV) system without celecoxib we could not obtain a CL spectrum since the emission was very weak. On the other hand, the emission peaks of both Na_2SO_3–Ce(IV)–Tb(III) and Na_2SO_3–Ce(IV)–Tb(III)–celecoxib systems are located at around 490 and 545 nm which is the characteristic fluores-

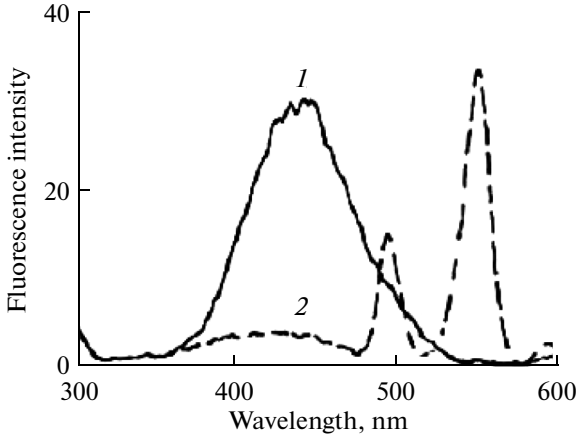


Fig. 2. Fluorescence emission spectra of celecoxib (*1*) and Tb(III)–celecoxib (*2*). $\lambda_{ex} = 270$ nm, 7×10^{-4} M Tb(III), 0.5 μg/mL celecoxib.

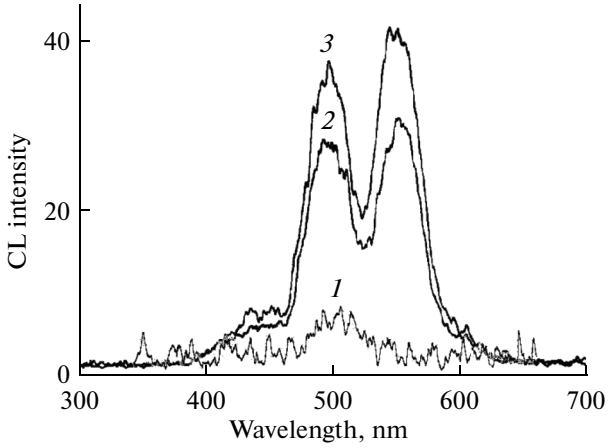


Fig. 3. CL spectra of Ce(IV)–Na_2SO_3–celecoxib (*1*), Ce(IV)–Na_2SO_3–Tb(III) (*2*) and Ce(IV)–Na_2SO_3–Tb(III)–celecoxib (*3*) obtained by continuous flow of reagents: 1.6×10^{-3} M Ce(IV) and 0.032 M H_2SO_4 in one line and 1.0×10^{-3} M Na_2SO_3 together with 8×10^{-4} M Tb(III) or 2 μg/mL celecoxib or Tb(III)–celecoxib in the other line.

cence peaks of terbium. This clearly indicates that the emitting species is excited Tb(III), and there must be energy transfers in the reaction system.

We also studied the composition of celecoxib–Tb(III) complex using mole ratio method. The result (Fig. 4) shows that the complex has a 2 : 1 stoichiometry. Based on these considerations, a sensitized CL mechanism can be proposed as follows [35]:

$$SO_3^{2-} + H^+ \longrightarrow HSO_3^-$$

$$Ce(IV) + HSO_3^- \longrightarrow Ce(III) + HSO_3^{\bullet}$$

$$2HSO_3^{\bullet} \longrightarrow S_2O_6^{2-} + 2H^+$$

$$S_2O_6^{2-} \longrightarrow SO_4^{2-} + SO_2^*$$

$$SO_2^* + (Tb{-}celecoxib)^{3+} \longrightarrow SO_2 + (Tb{-}celecoxib^*)^{3+}$$

$$(Tb{-}celecoxib^*)^{3+} \longrightarrow (Tb^*{-}celecoxib)^{3+}$$

$$(Tb^*{-}celecoxib)^{3+} \longrightarrow (Tb{-}celecoxib)^{3+} + h\nu \text{ (490 and 545 nm)}.$$

Optimization of chemical conditions. In order to obtain the maximum sensitivity for the determination of celecoxib, the effects of several analytical variables such as the concentration of Na_2SO_3, Ce(IV), H_2SO_4 and Tb(III)on the CL intensity were investigated. The effect of Na_2SO_3 concentration on the CL intensity was examined in the range of 2.5×10^{-4} to 1.0×10^{-3} M. According to the results (Fig. 5a) increasing Na_2SO_3 concentration enhanced the CL intensity, however, the excess Na_2SO_3 decreased the CL emission. The maximum CL intensity was obtained at 5×10^{-4} M Na_2SO_3; therefore, this amount was used as optimum concentration.

In order to study the effect of Ce(IV) concentration, solutions with different concentrations of $Ce(SO_4)_2 \cdot 4H_2O$ were prepared over the range of 5×10^{-4} to 1.5×10^{-3} M. According to the results (Fig. 5b), the maximum CL response was obtained for 8×10^{-4} M Ce (IV). At lower Ce(IV) concentrations the number of excited intermediates decreased and the response diminished.

The influence of sulfuric acid concentration in Ce(IV) solution on the CL signal was also investigated. As shown in Fig. 5c, the CL signal increased until 0.016 M and decreased in higher concentrations. The decrease in CL intensity at low concentrations is probably due to hydrolysis of Ce(IV) forming cerium hydroxide. Therefore, 0.016 M sulfuric acid was used for further work.

The effect of Tb(III) concentration in the range of 2.0×10^{-5} to 8.0×10^{-4} M on the CL intensity was also examined (Fig. 5d). The CL intensity increases by increasing the Tb(III) concentration up to 7.0×10^{-4} M and then remains constant. Hence, this concentration was used for further work.

Finally the effect of different surfactants (SDS, AOT, DDABr and CTAB) at various concentrations on the CL system was investigated. The results showed that none of these surfactants can make an enhancement in related CL intensity, so we avoided using them.

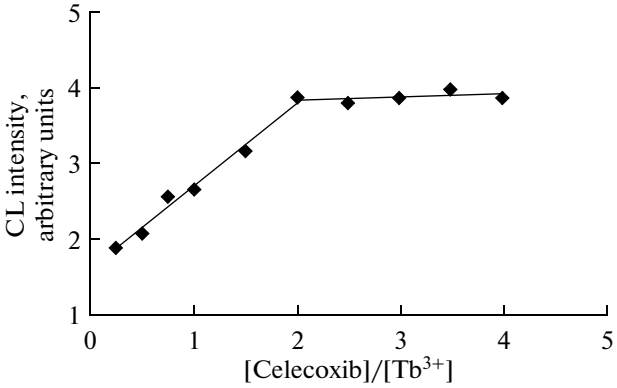


Fig. 4. The variation of CL intensity as a function of celecoxib–Tb(III) mole ratio.

Analytical application of the CL system. Under the optimum conditions described above, the analytical figures of merit for the determination of celecoxib was investigated. The CL response was found to be linear in the concentration range of 0.01–0.15 μg/mL with a limit of detection (**LOD**) (3*s*) of 2.5 ng/mL. The regression equation was $\Delta I = 72.91c + 2.67$, $R^2 = 0.9964$, where $\Delta I = I - I_0$ is the difference between CL intensity in the presence of celecoxib (I) and in its absence (I_0), and c is concentration of celecoxib in μg/mL. The relative standard deviation (**RSD**) was obtained to be 2.0 and 2.5% for five determinations of 0.05 and 0.075 μg/mL celecoxib, 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 celecoxib quantification is showed in Table 1. As can be seen, the developed method has a lower LOD than most of other methods. Moreover, it is very simple and inexpensive.

Table 1. Comparison of the developed CL method for the determination celecoxib with some previously published methods

Method	LOD, ng/mL	Linear range, μg/mL	Reference
Fluorescence	40[a]	0.13–2.0	[8]
	60[b]	0.19–2.32	
Fluorescence	7.29	0.1–4.0	[9]
UV	260	1–20	[6]
LC	12.5[c]	0.0125–0.15	[12]
LC–MS	20	0.05–1	[14]
LC–MS–MS	10[c]	0.01–2	[16]
CL	2.5	0.01–0.15	This work

[a] In ethanol. [b] In acetonitrile. [c] LOQ.

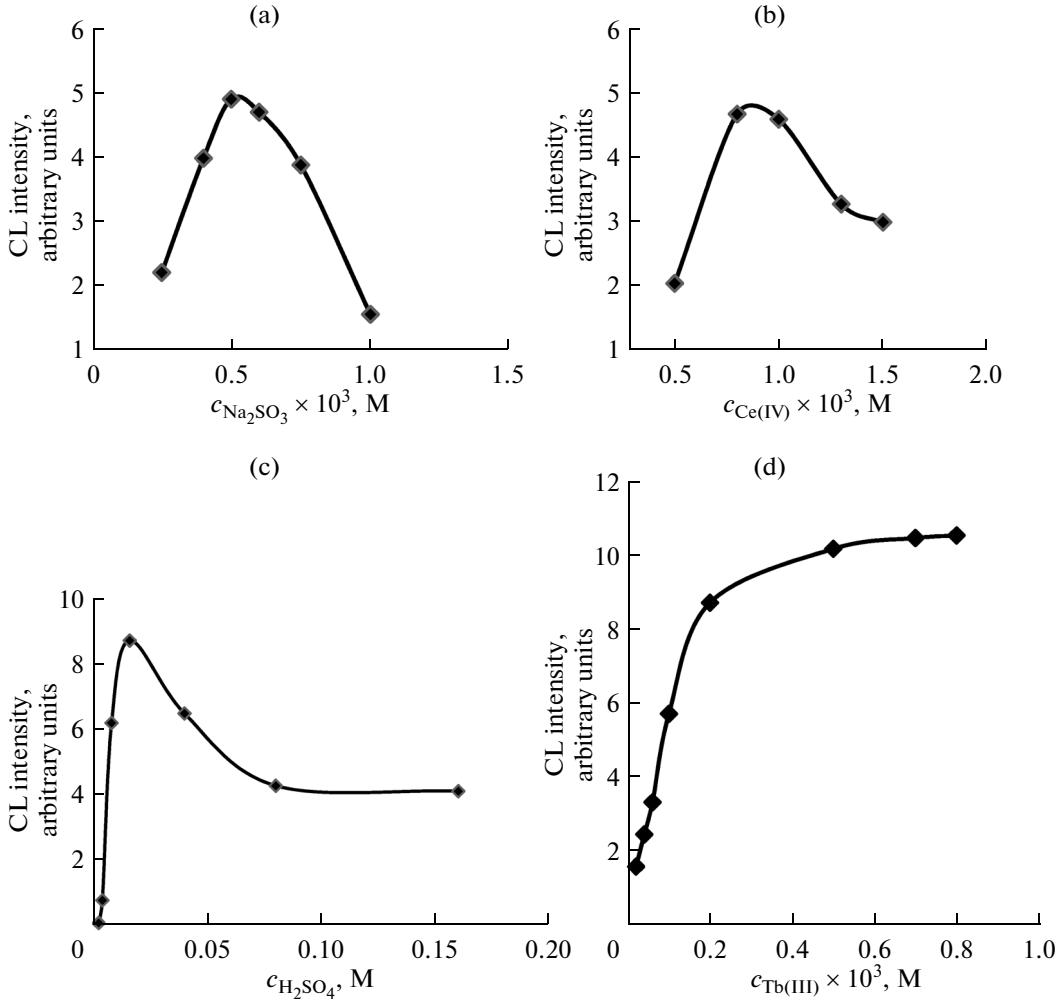


Fig. 5. Optimization of the CL reaction conditions: (a)—effect of Na_2SO_3 concentration: 8×10^{-4} M Ce(IV), 6×10^{-3} M H_2SO_4, 2×10^{-4} M Tb(III), 0.1 μg/mL celecoxib; (b)—effect of Ce(IV) concentration: 5×10^{-4} M Na_2SO_3, other conditions are as in (a); (c)—effect of H_2SO_4 concentration: 8×10^{-4} M Ce(IV), other conditions are as in (b); (d)—effect of Tb(III) concentration: 0.016 M H_2SO_4, other conditions are as in (c).

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 celecoxib were investigated. The tolerable concentration ratios for interferences with relative error of <5% were over 2000 for Na^+, Cl^-, glucose, sucrose, glycine, 1700 for SDS, 1500 for lactose, 1000 for K^+, Zn^{2+}, Ca^{2+}, Mg^{2+}, NO_3^-, 400 for Cu^{2+}, 300 for ascorbic acid, L-cysteine, 200 for Fe^{3+}, vitamins B_1 and B_2 and 50 for uric acid. As can be seen, the amounts of most potentially interfering species in plasma are below their tolerable levels or can be decreased with diluting, so there would be no interferences from these species in celecoxib determination. These results demonstrate that the method possesses a good selectivity for the determination celecoxib in pharmaceutical and biological samples.

Analytical applications. *Determination of celecoxib in pharmaceutical samples.* Celecoxib was satisfactorily determined in two different pharmaceutical brands (A: Celexib, Darou Pakhsh, Iran and B: Celebrex, Pharmacia, USA) by using the proposed method. The results obtained and the labeled contents are given in Table 2. Statistical analysis of these results using Student *t*-test shows that no significant differences between labeled and determined contents of celecoxib are observed.

Determination of celecoxib in human plasma samples. The present method was easily applied to the determination of celecoxib in spiked human plasma. The previously described procedure for deproteinization in "Experimental" was found to be necessary, and diluting processes were done for all the spiked serum samples to make the final concentrations of celecoxib in the linear range. Also in order to avoid matrix

Table 2. Results (μg/mL) for the determination of celecoxib in spiked plasma samples

Serum sample	Added	Found [a]	Recovery, %	*t*-statistic[b]
1	0.30	0.29 ± 0.01	97 ± 3.4	2.5
	0.50	0.47 ± 0.02	95 ± 4.4	1.73
	0.70	0.67 ± 0.02	95 ± 3.0	3.46
2	0.30	0.31 ± 0.01	104 ± 4.8	0.80
	0.50	0.49 ± 0.02	99 ± 4.3	0.86
	0.70	0.71 ± 0.02	101 ± 2.5	1.15

[a] Mean ± standard deviation, $n = 3$; [b] t-critical = 4.3 for $n = 2$ and $P = 0.05$.

Table 3. Results for determination of celecoxib in pharmaceutical samples (capsules)

Sample	Labeled amount, mg/capsule	Recovery, %	RSD, %	*t*-statistic[a]
A	100	100	2.2	0.6
B	200	103	3.4	1.9

[a] t-critical = 4.3 for $n = 2$ and $P = 0.05$.

effects, the standard addition method was applied. The obtained results are shown in Table 3.

Dissolution studies. The obtained release profiles for two celecoxib capsules at the previously mentioned conditions are shown in Fig. 6. An increase in celecoxib concentration in the dissolution medium with time was observed for both capsules. After 60 min, more than 90% of the drug in both tested capsules was dissolved in the medium. Pharmacopoeias very frequently use $t_{85\%}$ parameter as an acceptance limit of the dissolution test. US–FDA guidance for slowly dissolving or poorly water soluble product suggested that 85% ($t_{85\%}$) of labeled amount of drug should release within 60 min [36]. In this study, celecoxib capsules satisfied this criterion.

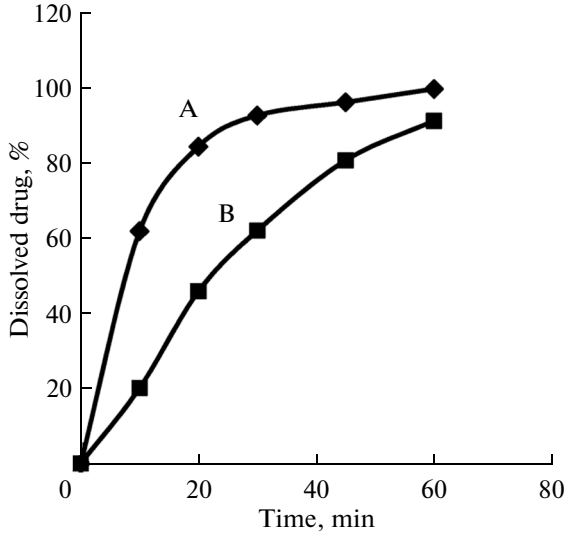


Fig. 6. Fraction of the dissolved drug depending on time in the dissolution studies of capsules of celecoxib (brands A and B).

* * *

A lanthanide sensitized chemiluminescence method was developed for the determination of celecoxib. Under the optimum condition, the CL intensity was proportional to the concentration of celecoxib. The possible mechanism of the chemiluminescence is also proposed. The proposed CL method has good linearity, high sensitivity and good reproducibility, and can be applied to determine celecoxib in pharmaceutical products and human plasma samples.

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