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Short communication

Sensitive determination of ketoprofen using flow injection with chemiluminescence detection

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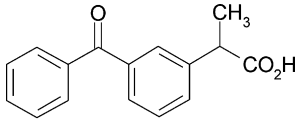
Abstract

A rapid chemiluminescence method is described for the determination of ketoprofen by combining the flow injection technique and its sensitizing effect on the weak chemiluminescence reaction between sulfite and acidic permanganate. The optimum conditions for the chemiluminescence emission were intensity. A mechanism for the chemiluminescence reaction has been proposed on the basis of chemiluminescent spectra. Ketoprofen can be determined over the concentration range of 5.0×10^{-8} to 3.0×10^{-6} mol/L with a correlation coefficient of 0.9999 and a detection limit of 2.0×10^{-8} mol/L (3σ). The relative standard deviation (R.S.D.) for 15 repetitive determinations of 1.0×10^{-6} mol/L ketoprofen is 0.8%. The utility of this method was demonstrated by determining ketoprofen in capsules and human urine sample.

Keywords: Ketoprofen determination; Chemiluminescence; Flow injection analysis; Permanganate; Sulfite

1. Introduction

Ketoprofen [(*RS*)-2-(3-benzoylphenyl)propanoic acid], an aryl propionic acid derivate, is a potent nonsteroidal anti-inflammatory agent that also has analgesic and antipyretic activity [1]. It has been widely used for the treatment of inflammatory diseases and musculoskeletal injury [2].



ketoprofen

Several methods have been reported for the quantitative determination of ketoprofen, including electrochemical [3–6], UV–vis spectrophotometry [7,8], thin-layer chromatography [9], liquid chromatography [10], capillary electrophoresis [11] and electrospray ionization mass spectrometry (ESI-MS) [12]. Flow-injection analysis (FIA) is a useful analytical technique since it is fast, cheap, accurate and precise, and had very wide applications in many areas. Aboul-Enein et al. developed a simple, sensitive and fast flow-injection analysis method with UV-detection method for the determination of ketoprofen in pharmaceuticals [13]. Linear calibration curves were obtained in the rage of 1.6×10^{-6} and 1.7×10^{-4} mol/L. Limit of detection were 1.7×10^{-6} mol/L (S/N = 3.3). Hasan Basan et al. have conducted a different study in that they report quantitative determination ketoprofen in gels and ampules by using flow-injection UV spectrophotometry and HPLC [14]. The detection limits were 1.7×10^{-6} and 1.2×10^{-6} mol/L (0.436 and 0.303 μg/mL). Analytical signal of the ketoprofen was linear in the concentration range of 2.9×10^{-5} to 2.9×10^{-4} mol/L (7.5–75 μg/mL). The method had a relatively high sampling rate, 85 h^{-1}, compared to conventional HPLC method, 15 h^{-1}. In another study conducted by Sánchez-Dasi et al., ketoprofen in pharmaceuticals was determined using flow injection Fourier transform infrared spectrometry [15]. This method provides a simple and useful way for the determination of ketoprofen in pharmaceuticals. It provided a 3σ limit of detection of 1.57×10^{-4} mol/L (0.04 mg/mL).

The aim of this work is to develop a simple and rapid method for the determination of ketoprofen that does not require sophisticated instruments but gives results comparable to those obtained by the existing optical methods. The method described is based on the sensitizing effect of ketoprofen on the chemiluminescence (CL) intensity emitted from the reaction of sulfite with acidic $KMnO_4$ and it was applied to the determination

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of ketoprofen. Analytical method applying CL combined with flow injection technique shows the advantages of high sensitivity, wide dynamic range and simple instrumentation [16], and has been found extensive application on in different fields [17,18]. To our best knowledge, this paper, which describes the first application of FIA-CL to the determination of ketoprofen. This method is simple and less expensive in comparison to the above-mentioned techniques and at the same time, offers a good accuracy, high speed and precision and has been used to determine ketoprofen in capsules and human urine sample. It can be regard as a basis for the development of an HPLC-CL method for the determination of ketoprofen in biological fluids.

2. Experimental

2.1. Reagents

All solutions were prepared from analytical-reagent grade materials in twice-distilled water. Ketoprofen was obtained from Jiangsu Institute For Drug Control, Nanjing (China). $KMnO_4$ (Shanghai, China) was used as received. The working solution of 1.0×10^{-4} mol/L $KMnO_4$ was prepared daily by diluting the stock solution of 1.0×10^{-2} mol/L $KMnO_4$ with 1.0×10^{-3} mol/L sulfuric acid. The solution of 1.0×10^{-2} mol/L sodium sulfite was prepared daily.

2.2. Apparatus

The FIA-CL system used in this work is shown in Fig. 1. Two pumps of Luminescence Analyzer (IFFM-E, Remex Electronic Instrument Limited Co., Xi'an, China) were used to deliver flow streams. Polytrafluoroethylere (PTFE) tubing (0.8 mm i.d.) was used to connect all components in the flow system. The flow cell was a 10 cm length of spiral glass tubing (2.0 mm i.d.) and the distance between injection valve and flow cell was about 10 cm. The CL signal was detected by the photomultiplier tube (PMT) (CR-105, Hamamatsu, Beijing, China) placed near the flow cell and was recorded with a computer equipped with an A/D card. The wavelength of its max sensitivity of the PMT is 420 nm.

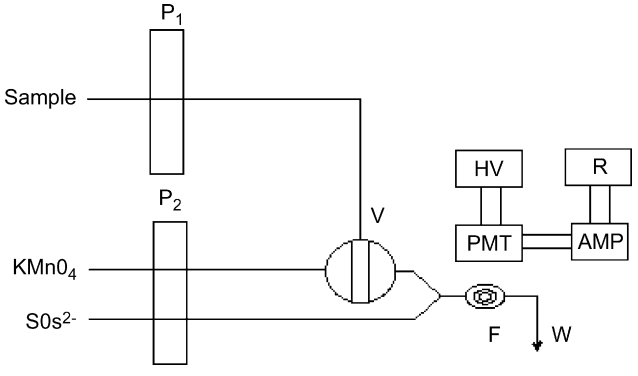

Fig. 1. Schematic diagram of the CL-FLA system for the determination of ketoprofen: P_1 and P_2, peristaltic pump; V, six-way injection valve; F, flow cell; W, waste solution; HV, high voltage; PMT, photomultiplier tube; AMP, amplitude; R, recorder.

The CL spectrum was obtained with a series interference filters by the static method. The filters were inserted between the sample cuvette and the photomultiplier tube (PMT).

2.3. Procedures

The CL reaction conditions were optimized with the following procedures. By keeping the valve in washing position, sulfite and acidic $KMnO_4$ solutions were continuously pumped into the manifold until the baseline was established on recorded signal. Flow rate was set at 2.5 mL/min for all lines. 80 μL ketoprofen solution was injected into the carrier stream (acidic $KMnO_4$) with the measurement frequency of 180 h^{-1} and allowing 20 s for sampling and washing. The content of ketoprofen was determined with the calibration plot of CL emission intensity versus ketoprofen concentration.

3. Results and discussion

3.1. Optimization of experimental variables

The CL emission that results from the redox reaction of $KMnO_4$ and Na_2SO_3 is not significant enough, which has relatively low sensitivity. When ketoprofen was added in this system, the CL emission increased. The significant increase indicated ketoprofen was a sensitive enhancer on the CL reaction of permanganate–sulfite. Furthermore, the emission intensity was proportional to the concentration of ketoprofen. The sensitizing effect of ketoprofen on the weak CL emission was also related to the pH value of solution and the concentrations of $KMnO_4$ and sulfite. Thus, a series of experiments were performed to optimize the conditions for the production of maximum CL emission.

The effect of acid contained in the solution on the CL emission was initially examined. The CL emission intensity of 1.0×10^{-6} mol/L ketoprofen–1.0×10^{-4} mol/L $KMnO_4$–5.0×10^{-3} mol/L Na_2SO_3 system in the presence of HCl, HNO_3, CH_3COOH, H_3PO_4 or H_2SO_4 at the same concentration was detected. The results indicated that the strongest CL emission occurred in acidic medium containing H_2SO_4. With the increasing concentration of H_2SO_4, the CL emission intensity increased and reached a maximum value at 1.0×10^{-3} mol/L (Fig. 2). Therefore, 1.0×10^{-3} mol/L H_2SO_4 was chosen as the acidic medium for the reduction of permanganate.

The effect of $KMnO_4$ concentration on CL intensity was examined in the range of 5.0×10^{-5} to 5.0×10^{-4} mol/L (1.0×10^{-3} mol/L H_2SO_4, 1.0×10^{-6} mol/L ketoprofen and 5.0×10^{-3} mol/L Na_2SO_3). The CL intensity increased with an increasing concentration of $KMnO_4$ and then reached a maximum value at the $KMnO_4$ concentration of 1.0×10^{-4} mol/L. Larger concentrations resulted in a decrease of the emission intensity. At the $KMnO_4$ concentrations higher than 1.0×10^{-4} mol/L, the emission intensity decreased probably owing to the permanganate absorbing the emitted light [19,20]. Therefore 1.0×10^{-4} mol/L $KMnO_4$ was used for subsequent work.

The effect of Na_2SO_3 concentration on the chemiluminescence intensity in the presence of 1.0×10^{-6} mol/L ketoprofen

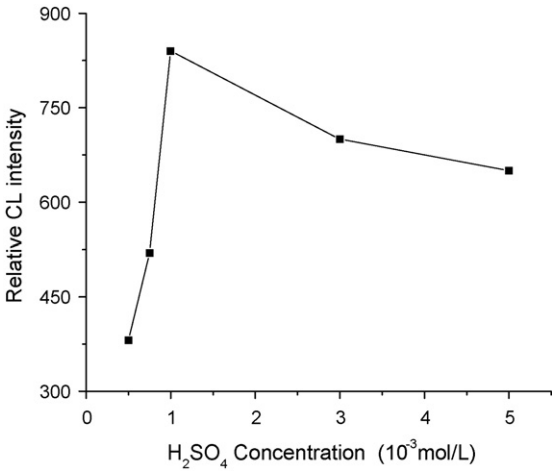

Fig. 2. Effect of H_2SO_4 concentration on CL intensity of 1.0×10^{-4} mol/L $KMnO_4$ + 1.0×10^{-6} mol/L ketoprofen + 5.0×10^{-3} mol/L Na_2SO_3.

and 1.0×10^{-4} mol/L $KMnO_4$ in 1.0×10^{-3} mol/L H_2SO_4 was studied. The result showed that the chemiluminescence intensity increased with the increase of the concentration of Na_2SO_3 when it was lower than 5.0×10^{-3} mol/L. Thus, 5.0×10^{-3} mol/L was chosen for the present work.

In flow injection analysis, the flow rate of each reagent stream is generally an important parameter. The solutions of 1.0×10^{-4} mol/L $KMnO_4$ in 1.0×10^{-3} mol/L H_2SO_4 and 5.0×10^{-3} mol/L Na_2SO_3 were introduced into the manifold at equal flow rate and the weak CL emission was continuously recorded as the baseline. The intensity of background emission was relevant to the flow rate. The signal intensity increased with the increasing flow rate, as it was expected from the increased mixing rate. The effect of the flow rate on the ketoprofen-sensitized chemiluminescence was the CL intensity also increased with the increasing flow rate. However, high flow rate led to much consumption of reagents and sample solutions but little gain in CL intensity and unstable CL signal. At a flow rate of 2.5 mL/min, the determination of ketoprofen, including sampling and washing, could be performed in 20 s, giving a sample measurement frequency of about 180 injections (60 samples) per hour. Thus, it was decided to supply the potassium permanganate and sulfite solution at 2.5 mL/min, respectively.

It is necessary to optimize the injection volume to achieve the desired sensitivity. Since the amounts of sample injected into the FIA system should be sufficient to permit effective CL reaction. The influence of the sample injection volume on the CL intensity was tested at 50, 60, 80, 100, 120 μL of 1.0×10^{-6} mol/L ketoprofen. The biggest relative chemiluminescence intensity and the best ratio of signal to noise were obtained when it was fixed between 80 and 100 μL. Thus, A 80 μL sample solution was injected into the carrier stream.

3.2. Kinetic characteristics of the CL reaction

The kinetic behavior of the CL reaction of ketoprofen–$KMnO_4$–Na_2SO_3 was studied with a static method. The CL reaction occurred immediately after mixing Na_2SO_3 with the solution containing $KMnO_4$ and ketoprofen and reached a maximum within 0.60 s. The CL reaction could be completed within 0.90 s after the reaction started. Thus, the CL reaction is very rapid. It is a flash-type emission and is apparently controlled by the mixing speed.

3.3. Analytical characteristics of ketoprofen

Under the optimum conditions mentioned above, the calibration curve was obtained for ketoprofen determination by plotting the CL signal versus ketoprofen concentration, which gave a linear range from 5.0×10^{-8} to 3.0×10^{-6} mol/L with a correlation coefficient of 0.9999. The detection limit was 2.0×10^{-8} mol/L, which was calculated as the amount of ketoprofen required to yield a net peak three times the standard deviation of the background signal (3σ). The relative standard deviation for 15 repetitive determinations of 1.0×10^{-6} mol/L ketoprofen was 0.8%, showing a good reproducibility.

3.4. Interferences

In order to assess the possible analytical applications of the CL method described, the influences of different metal ions and some excipients used in pharmaceutical preparations on the CL intensity were investigated by determining the CL emission of the solutions containing 1.0×10^{-6} mol/L ketoprofen and foreign species with continuously increasing concentration up to 1×10^{-4} mol/L. When the effect of each foreign species on the peak height was less than 5.0%, it was thought not to interfere the determination of ketoprofen. The obtained results in Table 1 showed that under the optimized conditions, some ions and the studied excipients in the tablets did not interfere the determination of ketoprofen. Therefore, this method can be suggested for the determination of ketoprofen in pharmaceutical preparations.

3.5. Determination of ketoprofen in capsules

The average content of capsules was calculated from the contents of 10 capsules (Ketoprofen Enteric-coated Capsules, which was composed of ketoprofen and some common excipients, made in Southwest Synthetic Pharmaceutical Corp., Chongqing, China, 50 mg per capsule). The contents were then finely homogenized and a portion of the power was weighed accurately, transferred into a 100 mL brown measuring flask and diluted to scale with water. The mixture was sonicated for 20 min to

Table 1
Tolerance to different substances in the determination of 1.0×10^{-6} mol/L ketoprofen

Species added	Concertration ratio to ketoprofen
K^+, Mg^{2+}, starch, dextrin	100
Ascorbic acid, Fe^{2+}, Fe^{3+}, Cu^{2+}	50
Na^+, Sn^{2+}, Pb^{2+}, Cr^{3+}, Ba^{2+}, Co^{2+}	20
Glucose, fructose, sucrose, uric acid	10

Table 2
Analytical results of ketoprofen in capsules

Sample	Added ($\times 10^{-7}$ mol/L)	Found[a] ($\times 10^{-7}$ mol/L)	R.S.D. (%)	Recovery[a] (%)	Content (mg/capsule)
1	0.0	1.9	1.9		49.3
	2.0	4.0	2.0	105.0	
	5.0	6.7	3.1	96.0	
2	0.0	4.2	2.6		51.0
	2.0	6.2	2.9	100.0	
	5.0	9.3	4.2	102.0	

[a] Average of three determinations.

aid dissolution and then filtered. An appropriate volume of the filtrate was diluted further with water so that the concentration of ketoprofen in the final solution was within its linear response range. The results for the determination of ketoprofen were given in Table 2. The relative standard deviations less than 4.2% with an average value of 2.8% and the recoveries between 96.0 and 105.0% with an average recovery of 100.8% were highly satisfactory and illustrated the good performance of the proposed method.

3.6. Determination of ketoprofen in human urine

The suggested method was used for the determination of ketoprofen in urine. A 1 mL of sample was mixed with 0.5 mL of acetonitrile and centrifuged for 5 min at 3000 rpm/min. Then the supernatant was fetched and the rest acetonitrile was blow-dried under a gentle stream of nitrogen gas. Finally, the prepared sample was diluted with distilled water directly or supplemented with ketoprofen to test the recovery of the method.

Two healthy female volunteers took 100 mg ketoprofen capsules orally in morning with empty stomach. After that, urine samples were collected in glass beakers after 4 h. The sample treatment and determination procedure were immediately applied to the urine samples without any delay. The results of the recovery studies of ketoprofen from human urine sample are shown in Table 3.

3.7. Possible CL mechanism

Earlier work suggested that electronically excited sulphur dioxide molecules were the emitters of the CL reaction of sodium sulfite with potassium permanganate. Meixner and Jaeschke proposed a following mechanism [21]:

$$HSO_3^- + MnO_4^- \rightarrow {}^\bullet HSO_3 + MnO_4^{2-}$$

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

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

$$SO_2^* \rightarrow SO_2 + h\nu$$

In this mechanism sulfite acts as a reductant to produce an excited molecule of sulfur dioxide, which emits radiation in the range of 300–550 nm [22]. The energy of the excited molecule can be easily transferred to a fluorescent molecule intentionally added in the system. Since the fluorophore has higher quantum efficiency, its application can produce stronger photon emission, facilitating the measurement [23]. Another way to enhance the CL emission is the application of intriguing examples of sensitizers. These compounds do not fluoresce but are capable of amplifying the CL emission intensity produced from the oxidation of sulfite. For example, 3-cyclohexylamino-propansulfonic (CAPS) is not a fluorophore; it can enhance the CL emission intensity from the oxidation of sulfite by $KMnO_4$. The sensitizing effect of CAPS is attribute to the presence of the cyclohexyl ring [24].

Since ketoprofen does not exhibit fluorescence in acidic medium and has not the cyclohexyl structure, the mechanism of sensitizing effect may differ from those of fluorescent compounds and CAPS. Acidic $KMnO_4$ is known as oxidant and can react with many organic compounds [25]. Thus it is possible ketoprofen is oxidized by $KMnO_4$. On the other hand, There is no fluorescence of ketoprofen $KMnO_4$–H_2SO_4 system indicate that the oxidized of ketoprofen may not be the emission emitters.

The CL spectrum of $KMnO_4$–Na_2SO_3–H_2SO_4 system showed one emission profile extending from 490 to 620 nm with maximum emission intensity at about 535 nm (Fig. 3a), which was similar to those measured with interference filters by Stauff and Jaeschke [26]. According to the suggestion reported in [21,22,26], the emitter was the excited sulfur dioxide. Thus, the oxidization product of HSO_3^- by the oxidized form of ketoprofen should be $HSO_3^\bullet$ radical. Two $HSO_3^\bullet$ radicals then combined to produce $S_2O_6^{2-}$, which gave the excited intermediate product SO_2* with an emission when it returned to its ground state [22].

Table 3
Results of ketoprofen determinations in urine

Human urinee sample no.	Content ($\times 10^{-7}$ mol/L)	Added ($\times 10^{-7}$ mol/L)	Found[a] ($\times 10^{-7}$ mol/L)	R.S.D. (%)	Recovery (%)
1	1.0	2.0	2.9	3.8	95.0
2	2.1	2.0	3.9	4.7	90.0

[a] Average of three determinations.

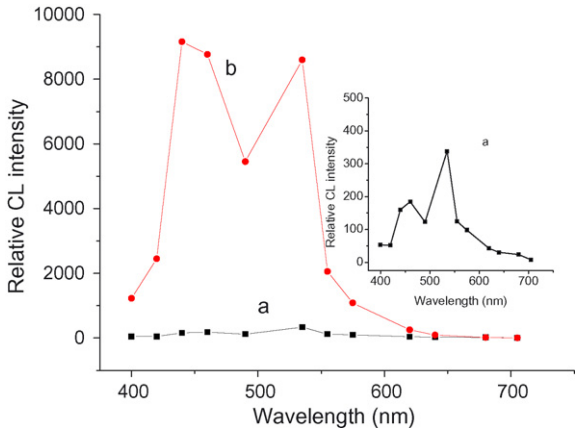

Fig. 3. CL spectra of $KMnO_4$–Na_2SO_3–H_2SO_4 (a) and ketoprofen–$KMnO_4$–Na_2SO_3–H_2SO_4 (b).

In ketoprofen–$KMnO_4$–Na_2SO_3–H_2SO_4 system, the CL spectrum showed two bands around 490–620 nm and 400–490 nm, respectively (Fig. 3b). The new maximum CL emission intensity occurred at about 440 nm, coinciding with one CL emission of noscapine–$KMnO_4$–Na_2SO_3–H_2SO_4 system of our previous work [27]. In our previous work we thought this CL emission intensity occurred at 440 nm might be due to the excited oxidized noscapine. But it could not explain the same CL emission in ketoprofen–$KMnO_4$–Na_2SO_3–H_2SO_4 system. Thus in ketoprofen–$KMnO_4$–Na_2SO_3–H_2SO_4 system, SO_2* and the excited [ketoprofen]$_{ox}$–manganese complex* might be the emitters. The mechanism of ketoprofen–$KMnO_4$–Na_2SO_3–H_2SO_4 system could be expressed as follows:

$$\text{ketoprofen} + MnO_4^- \rightarrow [\text{ketoprofen}]_{ox}^+ + \text{manganese complex}$$

$$[\text{ketoprofen}]_{ox}^+ + HSO_3^- \rightarrow HSO_3^\bullet + [\text{ketoprofen}]_{ox}^\bullet$$

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

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

$$SO_2^* \rightarrow SO_2 + h\nu\,(535\,\text{nm})$$

$$[\text{ketoprofen}]_{ox}^\bullet + \text{manganese complex} \rightarrow [\text{ketoprofen}]_{ox}\text{–manganese complex}^*$$

$$[\text{ketoprofen}]_{ox}\text{–manganese complex}^* \rightarrow [\text{ketoprofen}]_{ox}\text{–manganese complex} + h\nu\,(440\,\text{nm})$$

4. Conclusions

A flow injection CL method for the determination of ketoprofen has been well established based on the strong sensitizing effect on the weak CL reaction between sulfite and acidic $KMnO_4$. The sensitizing effect of ketoprofen on the reaction is due to the excited [ketoprofen]$_{ox}$–manganese complex* and SO_2*. Based on the sensitizing effect a CL method for determination of ketoprofen is purposed. This method is simple, highly sensitive and selective, and can be satisfactorily used in the determination of ketoprofen in practical sample.

Acknowledgements

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