

Chemiluminescence Determination of Sodium New Houttuynonate in Pharmaceutical Preparations Based on Tween 80—Rhodamine B System

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Received: 1 September 2006 / Accepted: 13 November 2006 / Published online: 12 December 2006
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Abstract Strong chemiluminescence (CL) emission was observed when sodium new houttuynonate (SNH) was mixed with Tween 80 in sulfuric acid medium in the presence of rhodamine B. Based on this phenomenon, a sensitive flow injection-CL method for the determination of SNH was developed. Under the optimum conditions, the CL emission is linearly with SNH concentration in the range 8.0–4000 ng mL⁻¹, with a detection limit of 2.7 ng mL⁻¹ (3 σ). As a preliminary application, the proposed method was successfully applied to the determination of SNH in pharmaceutical preparations. The possible CL mechanism was also discussed in this paper.

Keywords Sodium new houttuynonate · Tween 80 · Rhodamine B · Chemiluminescence

Introduction

Sodium new houttuynonate (SNH, sodium lauroyl- α -hydroxyethyl sulfonate) is a novel antimicrobial medicine for clinical practice in recent years (the chemical structure is shown in Fig. 1). Previous studies showed that houttuynonate

homologues (HOU-C_n) had immunoregulatory activities and typical adjuvanticity, and could improve the immune ability of mice and inhibit the growth of staphylococcus aureus, bacillus subtilis and pneumococci [1–3]. And it was reported that the immunoregulation and antibacterial activity of SNH were higher and stronger than that of sodium houttuynonate [4, 5].

Because of its potential pharmaceutical effects on health, the development of a simple, rapid and sensitive method for the determination of SNH in pharmaceutical preparations would therefore be highly desirable. HPLC method has been successfully applied to the determination of SNH in injections [6, 7]. Though HPLC method is accurate and specific, it suffered from the disadvantages of low sensitivity, narrow linear range and time-consuming.

Chemiluminescence (CL) has been established as a valuable detection technique with advantages including low detection limits, wide linear range, high analytical frequency and simple instrumentation [8]. In recent years, it was reported that weak CL emission could be produced by mixing the sulfite solution with Tween 80 in acidic media, and this weak CL emission could be greatly enhanced by introducing rhodamine dyes to the solution. The above CL system is simple and sensitive, and has been applied to the sensitive determination of sulfite [9], analgin, vitamin K₃ [10], and ethamsylate [11].

In recent studies, we observed that weak CL emission could be produced by mixing SNH with Tween 80 in sulfuric acid medium, and the CL intensity could be dramatically enhanced with the addition of rhodamine B to the reaction solution. Based on this phenomenon, a simple, rapid and highly sensitive flow injection CL method for the determination of SNH was proposed. The CL emission is linearly with SNH concentration in the range 8.0–4000 ng mL⁻¹ with a detection limit of 2.7 ng mL⁻¹ (3 σ). The method was

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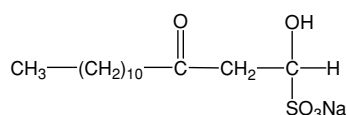


Fig. 1 Chemical structure of sodium new houttuifonate

successfully applied to the determination of SNH in pharmaceutical preparations.

Experimental

Apparatus

The flow system employed in this work is shown in Fig. 2. A peristaltic pump (Baoding Longer Precision Pump Co. Ltd., China) was used to deliver all streams at a flow rate (per tube) of 2.2 mL min^{-1} . PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. A $100 \mu\text{L}$ sample solution was injected into the carrier stream by a six-way injection valve. The flow cell was made by coiling a 10 cm length of glass tube (2.0 mm i.d.) into a spiral disk shape with a diameter of 2 cm and placed close to the window of the CR-150 photomultiplier tube (PMT, operated at -1000 V , Hamamatsu, Tokyo, Japan). The CL emission produced in the flow cell was detected by using a computerized BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Academia Sinica, Beijing, China). Data acquisition and treatment were performed with BPCL-K software running under Windows XP.

The CL spectrum was achieved with a set of interference filters, which were set between the mixing coil and PMT. Flow injection method described below was used to obtain the CL emission at different wavelength bands (400–745 nm).

Reagents

All the reagents were of analytical-reagent grade and double-distilled water was used throughout the experiment. SNH

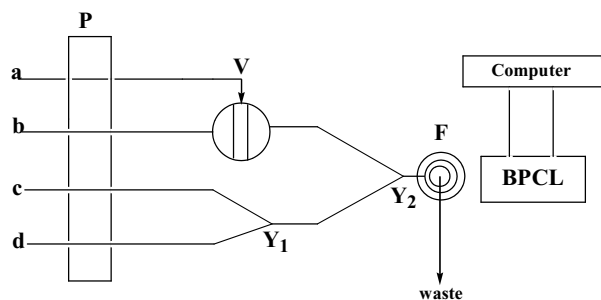


Fig. 2 Schematic diagram of the flow-injection CL system used for the determination of sodium new houttuifonate. (a) sample solution; (b) Na_2CO_3 carrier; (c) rhodamine B; (d) Tween 80; P, peristaltic pump; Y_1 , Y_2 , three-way junction; V, injection valve; F, CL flow cell; BPCL, BPCL ultra-weak luminescence analyzer

was obtained from Qinghai Xiadu Pharmaceutical Co., LTD; Tween 80 was obtained from Tianjin Chemical Reagent Plant; Rhodamine B was obtained from Shanghai Chemical Reagent Co. LTD. Rhodamine 6G was obtained from Sigma. A stock solution of SNH (0.4 mg mL^{-1}) was prepared by dissolving an appropriate amount of SNH in warm water and diluting to the mark with water. The stock solution was stored in the refrigerator at 4°C . A stock solution of 0.1 g mL^{-1} Tween 80 was prepared in water. A 5.0 mmol L^{-1} rhodamine B stock solution was prepared by dissolving 0.240 g rhodamine B in 100 mL water. Stock solutions of 0.1 mol L^{-1} sodium carbonate and 2.0 mol L^{-1} sulfuric acid were also prepared, respectively.

Procedure for flow injection analysis

The flow injection assembly is shown in Fig. 2, flow lines were inserted into the sample solution, Na_2CO_3 , rhodamine B and Tween 80 solution, respectively. The Tween 80 solution stream (containing 0.1 mol L^{-1} H_2SO_4 solution) was firstly merged with rhodamine B solution at Y_1 . After the valve was switched to the position of injection, a carrier stream (Na_2CO_3 solution) pushed $100 \mu\text{L}$ of sample solution to the mixing port Y_2 , where they met the mixture stream (rhodamine B + Tween 80) to produce strong CL emission. The concentration of the sample was quantified by the CL intensity of the system.

Procedure for pharmaceutical preparations

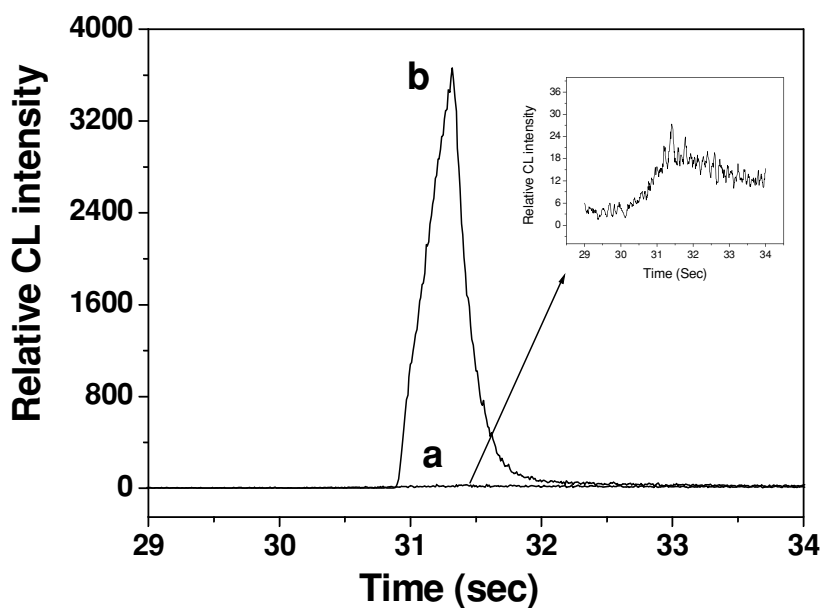
The SNH injection was purchased locally, which was manufactured by Guangdong YongKang Pharmaceutical Co., Ltd. The injection is an aqueous solution of SNH with a concentration of 10.0 mg of SNH in 5 mL solution. The SNH injection solution was diluted appropriately with redistilled water so that the final sample concentration was within the dynamic range, and then analyzed according to the procedure described above.

Results and discussion

Kinetic profile of the CL reaction

Before carrying out the flow injection method, the batch method for the CL profiles was applied. As shown in Fig. 3, without any special CL reagent, upon mixing the SNH and Tween 80 solution in sulfuric acid medium, an ultra-weak CL was observed (Fig. 3a). Furthermore, when the rhodamine B was present in the above reaction system, a strong CL emission was recorded. From Fig. 3b, it can be seen that the proposed CL reaction was a flash type luminescence and the time interval between the start of CL and its maximum was

Fig. 3 CL-time profiles in the static system. (a) 2.5 mL 10.0 mg mL⁻¹ Tween 80 in 0.1 mol L⁻¹ H₂SO₄ + 0.5 mL 2.0 × 10⁻⁷ g mL⁻¹ SNH. (b) 2.5 mL 10.0 mg mL⁻¹ Tween 80 in 0.1 mol L⁻¹ H₂SO₄ + 2.5 mL 0.2 mmol L⁻¹ rhodamine B + 0.5 mL 2.0 × 10⁻⁷ g mL⁻¹ SNH. The high voltage was set at -500 V



only about 0.4 s, and then the CL intensity quickly decreased to the baseline.

Effect of surfactants

The utilization of organized surfactant assemblies as a CL reaction medium is currently of interest for improving the CL quantum efficiencies or energy-transfer efficiencies [12–14]. In order to investigate whether micellar media function effectively for the present CL system, some organized systems, including three non-ionic surfactants (Tween 20, Tween 80, Polyethylene glycol 600), one cationic surfactant (CTMAB) and one anionic surfactant (SDS) were added to the present system. As can be seen from Table 1, apart from Tween series, all other systems could not enhance the CL emission. As Tween 80 gave the stronger CL signal than Tween 20, therefore, Tween 80 was selected for the following study.

The effect of Tween 80 concentration on the CL intensity was studied at different concentrations from 2.5 to 20.0 mg mL⁻¹. and it was observed that the CL intensity increased with increasing the concentration of Tween 80, remained almost constant in the range of 7.5–10.0 mg mL⁻¹,

and decreased when the concentration of Tween 80 was >10.0 mg mL⁻¹. Hence, 10.0 mg mL⁻¹ Tween 80 was selected for the following experiments.

Selection of energy transfer reagent

In order to obtain a high sensitivity, some fluorescing compounds were selected as energy transfer reagents and their sensitizing effects on the present CL reaction were examined. As illustrated in Table 2, both rhodamine B and rhodamine 6G enhanced the CL signal tremendously, and they gave out the similar I/I_0 value (where I and I_0 are the CL signal in the presence and absence of SNH, respectively.). In the present CL system, rhodamine B was selected as the sensitizer because of its good water solubility. The effect of rhodamine B concentration on the CL intensity was investigated in the range of 0.0–0.5 mmol L⁻¹. As can be seen from Fig. 4, the CL intensity increased sharply with increasing the rhodamine B concentration up to 0.2 mmol L⁻¹, above which the CL

Table 1 Effect of some organized solutions on CL emission

Surfactants (10.0 mg mL ⁻¹)	Relative CL intensity
None	0
Cetyltrimethylammonium bromide	16
Sodium dodecyl sulphate	6
Polyethylene glycol 600	9
Tween 20	580
Tween 80	710

Table 2 Effect of different fluorophores on the CL emission

Fluorophore (0.2 mmol L ⁻¹)	I/I_0^a
None	1.1
Quinine	1.5
Riboflavine	1.4
Fluorescein sodium	1.1
Rhodamine B	23.4
Rhodamine 6G	24.2

^aWhere I and I_0 are the CL signal in the presence and absence of 2.0 × 10⁻⁷ g mL⁻¹ of SNH, respectively.

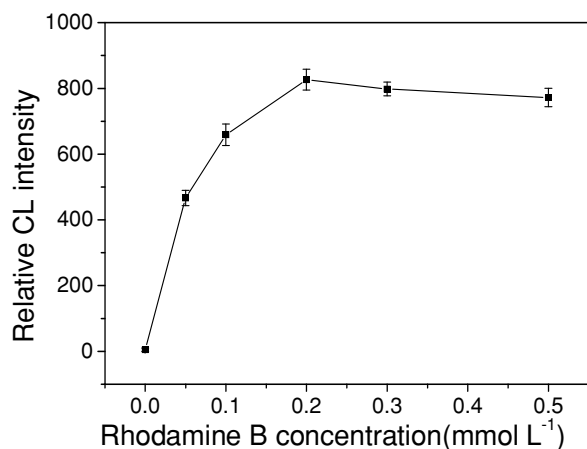


Fig. 4 Effect of rhodamine B concentration on the CL intensity. Tween 80, 10.0 mg mL⁻¹ in 0.1 mol L⁻¹ H₂SO₄; Na₂CO₃, 2.0 mmol L⁻¹; SNH, 2.0 × 10⁻⁷ g mL⁻¹

signal decreased slightly. Thus, 0.2 mmol L⁻¹ rhodamine B was chosen for further experiments.

Effect of acidic medium

In the present study, the CL emission could only be generated in acidic medium. The nature and concentration of the acid used in the reaction had a very strong influence on the CL emission. Therefore, four different acids (HClO₄, HCl, HNO₃, and H₂SO₄) of different concentration were tested in order to obtain the optimal conditions. H₂SO₄ produced the strongest CL signal and thus was selected as the acidic medium. The effect of the concentration of H₂SO₄ was studied in the range of 0.01–0.3 mol L⁻¹, and it was found that CL intensity increased H₂SO₄ concentration, was greatest at 0.1 mol L⁻¹, and thereafter decreased. Therefore, 0.1 mol L⁻¹ H₂SO₄ was chosen for further study.

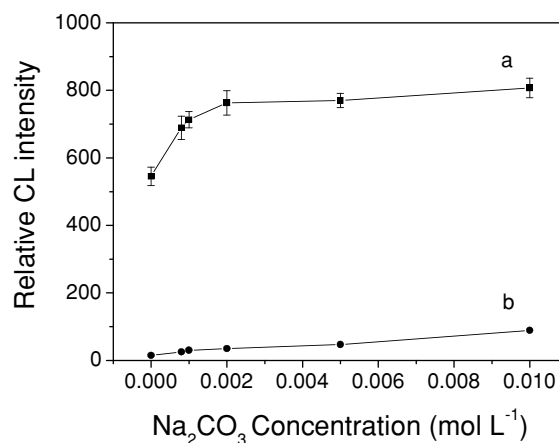


Fig. 5 Effect of Na₂CO₃ concentration on the CL intensity in the presence (a) and absence (b) of SNH. Tween 80, 10.0 mg mL⁻¹ in 0.1 mol L⁻¹ H₂SO₄; rhodamine B, 0.2 mmol L⁻¹; SNH, 2.0 × 10⁻⁷ g mL⁻¹

Selection of carrier solution

In the preliminary experiment, it was observed that the CL intensity could be increased in alkaline carrier stream. This can be explained by the reversible equilibrium reaction between SNH and sodium bisulfite (as shown in Scheme 1) in aqueous solution, and the equilibrium lies toward sodium bisulfite in basic media, which in turn leading to a strong CL emission. Several basic solutions, such as Na₂CO₃, NaOH and Na₃PO₄ were tested under the same conditions, and the results showed that they had no significant differences. In the present study, Na₂CO₃ was selected and the effect of Na₂CO₃ concentration was studied in the range of 0.0–10.0 mmol L⁻¹. As shown in Fig. 5, the CL intensity increased sharply with increasing Na₂CO₃ concentration from 0.0 to 2.0 mmol L⁻¹, and remained almost unchanged when Na₂CO₃ concentration was above 2.0 mmol L⁻¹, whereas the background signal was increased along the increase of Na₂CO₃ concentration at the same time. To compromise the

Scheme 1

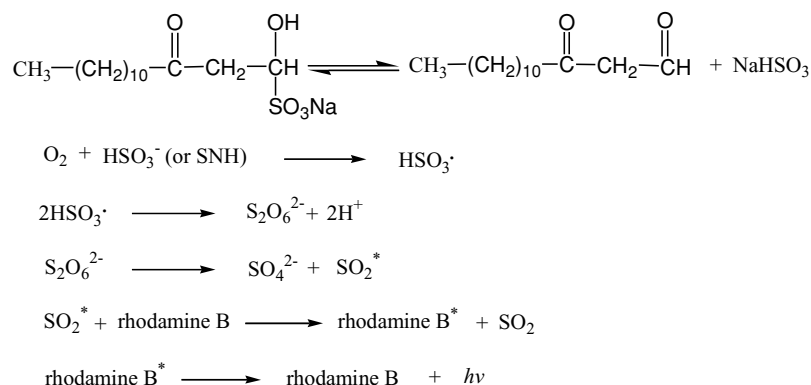
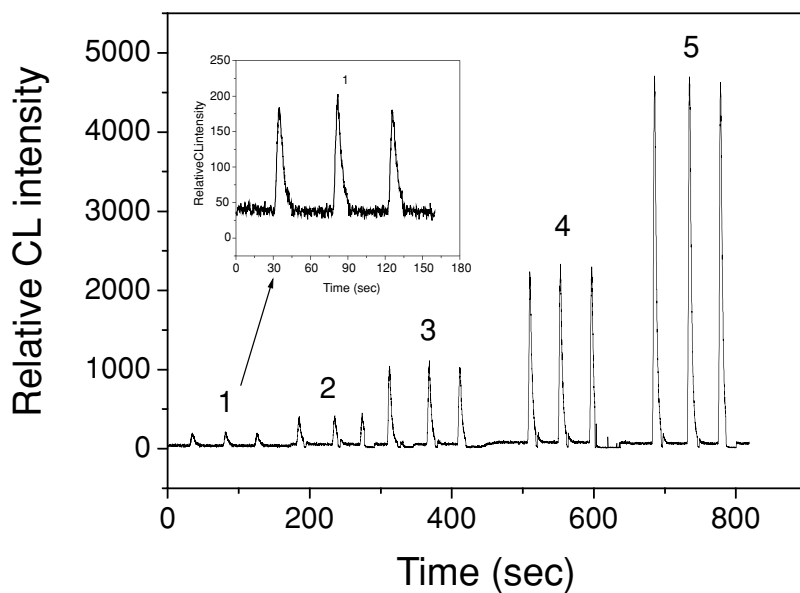


Fig. 6 The CL peaks obtained at different concentrations of SNH using flow injection method. The CL peaks of 1–5 were obtained at the concentration of 40, 100, 200, 500 and 1000 ng mL⁻¹, respectively. Other conditions are the same as those described in the procedure for flow injection analysis



signal to background ratio, 2.0 mmol L⁻¹ Na₂CO₃ solution was selected as carrier stream.

Effect of flow rate

The flow rates of the solutions are very important to the CL reaction. The effect of flow rate on the CL emission was studied over the range of 0.74–3.70 mL min⁻¹ under the optimum conditions described above. It was found that CL intensity increased with increasing flow rate, indicating a fast dynamic process of this reaction. As a compromise between reagent consumption and CL intensity, a flow rate of 2.2 mL min⁻¹ was chosen for the present CL system.

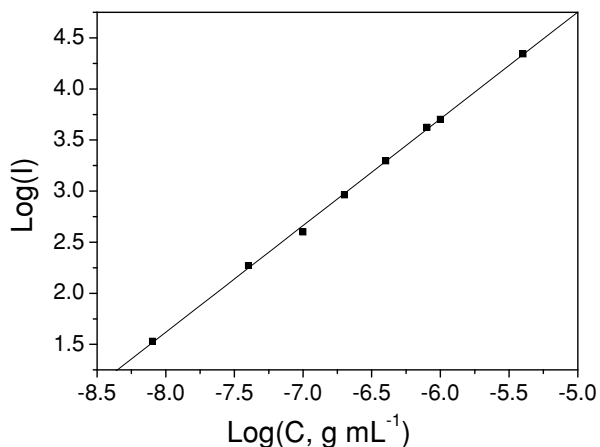


Fig. 7 A graph of log (*I*_{CL}) vs log (SNH concentration) over the entire dynamic range

Analytical characteristics

Under the selected conditions described above, the CL peak increased with increasing the SNH concentration (as shown in Fig. 6). The calibration graph of CL intensity (*I*, relative unit) vs SNH concentration (*C*, g mL⁻¹) was linear in the range of 8.0–4000 ng mL⁻¹ (as shown in Fig. 7) with a detection limit (3σ) of 2.7 ng mL⁻¹. The regression equation was *I* = 4.71*C* (*C*, ng mL⁻¹) – 16.8 with a correlation coefficient of 0.9975 (*n* = 8). The relative standard deviation for 20 and 200 ng mL⁻¹ of SNH in 8 replicate measurements are 3.6% and 6.4%, respectively.

Interference Studies

In order to assess the possible analytical application of the proposed method to dosage forms, the effect of foreign substances were studied by analyzing a standard solution of 0.4 μmol L⁻¹ SNH to which increasing amounts of interfering species were added. The tolerable concentration ratios with respect to 0.4 μmol L⁻¹ SNH for interference at ± 5%

Table 3 Tolerance limit of some foreign substances on the determination of SNH (0.4 μmol L⁻¹)

Substance	Mole ratio to SNH
K ⁺ , NH ₄ ⁺ , Zn ²⁺ , PO ₄ ³⁻ , NO ₃ ⁻ , starch, cyclodextrin, urea, citrate	≥ 1000
Glucose, sucrose, Co ²⁺ , Ca ²⁺	500
Al ³⁺ , Cu ²⁺ , Ni ²⁺ , thiamine, ascorbic acid	100
Oxalate	50
Fe ³⁺	1

Table 4 Results of the determination of SNH in injections^a

Sample	Amount labeled (mg)	This method ^b (mg)	UV method ^b (mg)
Injection 1	10	9.83 ± 0.31	9.91 ± 0.15
Injection 2	10	9.71 ± 0.23	9.87 ± 0.11

^aThe SNH injection was manufactured by Guangdong YongKang Pharmaceutical Co., Ltd and claimed containing 10.0 mg of SNH in 5 mL aqueous solution.

^bAverage of four measurements (± S.D.).

level was shown in Table 3. It can be seen that most common inorganic ions and organic substances have almost no effect on the determination of high concentration of SNH.

Sample analysis

In order to evaluate the validity of the proposed method, SNH in commercially available SNH injection was determined following the procedure described previously. As shown in Table 4, the results agree well with the labeled values. The reliability of the proposed method was also evaluated by comparing the results with those obtained from the UV spectrophotometry. No significant differences were observed between the both methods.

Mechanism of the chemiluminescence reaction

The oxidation of sulfite in acidic medium is a well known CL reaction and the analytical properties of the reaction have been reported with a variety of oxidants [12, 15–21]. The radical mechanism has been proposed for the CL oxidation of sulfite [12, 15, 17]. Because there is a reversible equilibrium reaction between SNH and sodium bisulfite in aqueous solution (shown in Scheme 1), SNH, lauroyl acetaldehyde and sodium bisulfite are both present in the solution, and the equilibrium lies toward lauroyl acetaldehyde and sodium bisulfite in basic media. To test whether the CL emission comes from the auto-oxidation of bisulfite, a 0.61 μmol L⁻¹ NaHSO₃ solution was introduced into the CL system, and a 1.4-fold higher CL signal was observed compared with the same concentration of SNH, indicating that the CL emission is bisulfite-involved. Moreover, acetaldehyde was also tested in the present system, and no CL emission was observed, indicating that lauroyl acetaldehyde cannot generate CL signal in the present system. Based on the above results, we thus believe that the CL emission of the present system is ascribed to the auto-oxidation of SNH and its decomposing product, sodium bisulfite.

Furthermore, the CL spectrum of the Tween 80—rhodamine B—SNH system was recorded by a series of interference filters (400–745 nm). The experimental results

showed that the maximum CL emission was at about 575 nm, which was in agreement with that of the fluorescence emission maximum of rhodamine B [22]. Thus, the luminophore could be ascribed to rhodamine B.

Based on the experimental results, we propose the mechanism of the present CL reaction as follows. In alkaline media, the equilibrium of SNH lies toward sodium bisulfite. Both SNH and sodium bisulfite are oxidized by dissolved oxygen to generate hydrogensulfite radicals (HSO₃·) [9, 22], and then two HSO₃· react to produce S₂O₆²⁻. S₂O₆²⁻ will give the excited intermediate product SO₂* [12, 17, 19]. The energy of SO₂* is easily transferred to rhodamine B, which corresponds to the peak at 575 nm. The possible mechanism stated above can be expressed as in Scheme 1.

Conclusions

In summary, a novel CL method has been proposed for the determination of SNH. The method was based on the auto-oxidation of SNH in acidic rhodamine B and Tween 80 surfactant micelles. It offers the advantages of simplicity, rapidity, high sensitivity and wide linear range, and has a potential application in pharmaceutical preparations and biological fluids.

Acknowledgements This work was supported by the Natural Science Foundation of Northwest University of China (04NW46). And the author thanks the Northwest University of China for startup funds.

References

- Ye XL, Li XG, Yuan LJ, Zhang BS (2006) Interaction between houthuyfonate homologues and erythrocyte plasma membrane of rabbit in vitro. *Colloids and Surfaces A: Physicochem Eng Aspects* 279:218–224
- Wang DY, Yu QH, Eikstadt P, Hammond D, Feng Y, Chen N (2002) Studies on adjuvanticity of sodium houthuyfonate and its mechanism. *Int Immunopharmacol* 2:1411–1418
- Wang DY, Noda Y, Zhou Y, Nitta A, Nabeshima T, Yu QH (2004) Effects of sodium houthuyfonate on phosphorylation of CaMK II, CREB and ERK 1/2 and expression of c-Fos in macrophages. *Int Immunopharmacol* 4:1083–1088
- Yuan LJ, Li XG, He HM (2004) Relativity of surfactivity and antibacterial motive of houthuyne analogies. *Acta Chin Med Pharmacol* 32(6):7–9
- Ye XL, Li XG, Yuan LJ, He HM (2005) Effect of the surface activity on the antibacterial activity of octadecanoyl acetal sodium sulfite series. *Colloids Surf A: Physicochem Eng Aspects* 268:85–89
- Zhang LH, Jing N (2005) Determination of sodium new houthuyfonate in injection by HPLC. *Chin Hosp Pharm J* 25(9):9845–9847
- Ji B, Wang D, Wang DK, Xue MY, Liu SP, Xu S, Zhuang R (2006) HPLC determination of sodium new houthuyfonate for injection and its related substances. *Chin J Pharm Anal* 26(1):86–88
- Garcia-Campana AM, Baeyens WRGE (2001) *Chemiluminescence in analytical chemistry*. Marcel Dekker, New York

9. Huang YM, Zhang C, Zhang XR, Zhang ZJ (1999) Chemiluminescence of sulfite based on auto-oxidation sensitized by rhodamine 6G. *Anal Chim Acta* 391:95–100
10. Huang YM, Zhang C, Zhang XR, Zhang ZJ (1999) Chemiluminescence analysis of menadione sodium bisulfite and analgin in pharmaceutical preparations and biological fluids. *J Pharm Biomed Anal* 21:817–825
11. Yang FZ, Zhang C, Baeyens WRGE, Zhang XR (2002) Determination of ethamsylate in pharmaceutical preparations based on an auto-oxidation chemiluminescence reaction. *J Pharm Biomed Anal* 30:473–478
12. Kato M, Yamada M, Suzuki S (1984) Flavin mononucleotide sensitized and polyoxyethylene (20) sorbitan trioleate micelle-enhanced gas/solution chemiluminescence for direct continuous monitoring of sulfur dioxide in the atmosphere. *Anal Chem* 56(13):2529–2534
13. Li Z, Feng ML, Lu JR (1998) KMnO_4 -Octylphenyl polyglycol ether chemiluminescence system for flow injection analysis of uric acid in urine. *Microchem J* 59:278–283
14. Lin J-M, Yamada M (2003) Microheterogeneous systems of micelles and microemulsions as reaction media in chemiluminescent analysis. *Trends Anal Chem* 22(2):99–107
15. Lin J-M, Hobo T (1996) Flow-injection analysis with chemiluminescent detection of sulphite using Na_2CO_3 - NaHCO_3 - Cu^{2+} system. *Anal Chim Acta* 323:69–74
16. Li B, Zhang Z, Zhao L, Xu C (2002) Chemiluminescence flow-through sensor for pipemidic acid using solid sodium bismuthate as an oxidant. *Anal Chim Acta* 459:19–24
17. Li B, Zhang Z, Wu M (2001) Flow-injection chemiluminescence determination of sulfite using on-line electrogenerated silver(II) as the oxidant. *Anal Chim Acta* 432:311–316
18. Li B, Zhang Z, Zhao L (2002) Flow-injection chemiluminescence detection for studying protein binding for drug with ultrafiltration sampling. *Anal Chim Acta* 468:65–70
19. Zheng X, Zhang Z (2002) Flow-injection chemiluminescence detecting sulfite with in situ electrogenerated Mn^{3+} as the oxidant. *Sens Actuat B* 84:142–147
20. Koukli II, Sarantonis EG, Calokerinos AC (1988) Continuous-flow chemiluminescence determination of sulphite and sulphur dioxide. *Analyst* 113:603–608
21. Pauls DA, Townshend A (1995) Sensitized determination of sulfite using flow injection with chemiluminescent detection. *Analyst* 120:467–469
22. Yang XF, Guo XQ, Zhao YB (2002) Novel spectrofluorimetric method for the determination of sulfite with rhodamine B hydrazide in a micellar medium. *Anal Chim Acta* 456(1):121–128