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CHEMILUMINESCENCE FLOW INJECTION ANALYSIS OF MENADIONE SODIUM BISULFITE BASED ON LUMINOL REACTION

Keywords: Chemiluminescence, menadione sodium bisulfite, luminol, Vitamin K_3

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ABSTRACT

A chemiluminescence(CL) flow system is described for the determination of menadione sodium bisulfite based on its repression on the chemiluminescence(CL) emission produced upon mixing a hexacyanoferrate(III) solution with an alkaline luminol solution in the absence of co-oxidizer. The system responds linearly to menadione sodium bisulfite concentration in the range 0-1 μg/mL with a detection limit (3σ) of 0.01 μg/mL. Relative standard deviation (RSD) of 0.16% for 0.4 μg/mL menadione sodium

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bisulfite (n=11). The system has been successfully applied to the determination of menadione sodium bisulfite in tablets and injections.

INTRODUCTION

Menadione (vitamin K_3) plays an important role in blood coagulation as a clotting factor and in bone-mineralization processes. Menadione sodium bisulfite (MSB) is a water-soluble form of menadione and is an effective dose in vitamin-K deficiency. Recently, growing efforts have been paid to this compound due to its potential anticancer activity [1-4].

Numerous methods for the determination of MSB have been published, for example spectrophotometry[5,6], electrochemistry [7-9], and fluorimetry [10-12], however, each has some drawback such as lack of sensitivity, selectivity or simplicity. It is now widely realized that chemiluminescence detection in analysis can be a means of achieving high sensitivity over a wide dynamic range with low detection limits. Most chemiluminescence methods to date are applications of well-know reactions such as luminol oxidation. In general an additional oxidizer is needed in the conventional luminol CL system; among various oxidizers, hydrogen peroxide is the most widely used. As with most chemiluminescent reactions, the presence of certain metal ions or metal -containing species ellicit CL from aqueous luminol solutions in the absence of oxidant, thus forming the basis for methods for the determination of the metal or metal-containing species[13-18]. In addition, the presence of reductants such as uric acid, cortisone, ascorbic acid, glutathione, cysteine, etc. have been found to similarly give rise to CL from the luminol system[19]. However, in this later case, a catalyst such as hexacyanoferrate(III) is necessary[19]. In these CL reactions, the postulated primary emitter is the 3-aminophthalate ion (3-AP)[16,19]. P-Ruiz et.al[20] recently reported a luminol-based FIA-CL method for the indirect determination of vitamin K_3 based on the photooxidation of ethanol, which is sensitized by vitamin K_3. The hydrogen peroxide thus formed was

monitored by the luminol – hematin CL reaction. There are some other CL systems such as the peroxyoxalate CL system[21-23] and the lucigenin CL system[24] for the determination of quinones, a family of which vitamin K_3 is a member.

Upon addition of MSB to a solution containing luminol and hexacyanoferrate(III), a decrease in the CL is observed. This observation forms the basis of a simple and rapid FIA-CL method for determination of MSB. This paper describes the optimization of this CL reaction system and summarizes the pertinent analytical parameters. This approach could also form the basis for development of a HPLC-CL method for the determination of MSB in biological fluids.

EXPERIMENTAL

Reagents

All the reagents were of analytical-reagent grade unless specified otherwise; doubly distilled water was used for the preparation of solutions. MSB solution(1000 μg/mL) was prepared. More diluted solutions were used immediately after preparation. Potassium hexacyanoferrate(III) was obtained from Chongqing Chemical Reagent Company. A 0.01 mol/L luminol solution was prepared by dissolving 1.772 g of luminol in 1000 mL of 0.01 mol/L NaOH.

Apparatus

The flow system employed in this work is shown in Fig.1. A peristaltic pump delivered all follow streams at a flow rate of 1.5 ml min^{-1} (per tube). PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. 70μl of sample solution was injected into water stream by a eight-way injection valve and then mixed with the reagent streams (a mixture of

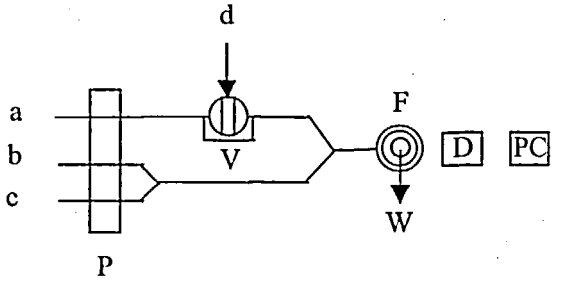

Fig.1. Schematic diagram of the flow system for the determination of MSB: (a): H_2O; (b): 50 μM potassium hexacyanoferrate(III); (c): 30 μM luminol in 0.4M NaOH; (d): sample; (D): detector; (PC): personal computer; (P): pump; (V): valve; (F): flow cell; (W): waste

hexacyanoferrate(III) solution and alkaline luminol solution). The emitted CL was collected with a photomultiplier tube (operated at –400V) of the Type IFFL-D Flow-Injection Chemiluminescence Analyzer (Reike, Xi'an). The signal was recorded using an IBM-compatible computer, equipped with a data acquisition interface. Data-acquisition and treatment were performed with REMAX software running under Windows 98. Chemiluminescence spectrum was monitored using a modified RF-540 fluorescence spectrometer (Shimazhu, Japan). UV spectrum was made on a Hitachi-3400 UV spectrophotometer (Hitachi, Japan).

Procedure

Procedure for calibration

Working standard solutions containing MSB in the range of 0-1 μg/mL were prepared by dilution of a concentrated fresh standard solution of MSB (1000μg/mL). The CL signal was measured by injecting 70 μl of working standard solution into the water carrier stream, which then joined the reagent

streams (a mixture of 50 μM hexacyanoferrate(III) solution and 30 μM luminol in 0.4 M NaOH solution). The CL emission intensities *versus* MSB concentration were used for the calibration.

Procedure for tablets

Ten tablets of MSB were weighed to obtain mean tablet weight, then ground to a homogenized powder; an accurately weighed portion of powder corresponding to 4 mg was then diluted to 100 mL with doubly distilled water, and then diluted 200-fold with doubly distilled water for a quantitative analysis.

Procedure for injections

Injection samples, each with a nominal content of 4 mg of MSB in 1 mL, were diluted to 100 mL with doubly distilled water, and then diluted 200-fold with doubly distilled water for the analysis.

RESULTS AND DISCUSSION

The characteristics of MSB inhibition of the light emission produced by luminol on oxidation by dissolved oxygen with hexacyanoferrate(III) catalysis

It is well known that the primary emitter in the luminol CL reaction is considered to be 3-aminophthalate (3-AP)[16,19]. In our experiments we examined the CL spectra by a modified RF-540 Fluorimeter, which showed only one peak at about 425nm (same as the maximum emission spectra of 3-AP).

In the present work, chemiluminescence kinetic characteristics of the CL reaction were studied in detail. It was found that the rate of the CL reaction in solution was very fast; from the reagent mixing to the peak maximum only 0.5 s was needed and it took about 5 s for the signal to reach zero again. In order to

study the role of MSB, we conducted several experiments in which reaction products were scanned with UV spectrophotometer. The results listed in Fig. 2, show clearly from curves d and e that in the experimental conditions MSB reacts with potassium hexacyanoferrate(III), leading to a decrease of potassium hexacyanoferrate(III) concentration. From curves a and c, we conclude that mixing of potassium hexacyanoferrate(III) and luminol in basic media leads to consumption of potassium hexacyanoferrate(III). As can be seen from curves b and c, the hypothesis that MSB reacts with potassium hexacyanoferrate(III) in the experimental conditions is further confirmed. Based on the above discussions, the possible mechanism of the CL reaction may be that menadione sodium bisulfite reacts with potassium hexacyanoferrate(III), leading to decrease of potassium hexacyanoferrate(III) concentration, the remaining potassium hexacyanoferrate(III) reacts with luminol to produce an electronically excited 3-aminophthalate, which corresponds to the peak at about 425nm.

Optimization of experimental conditions

Upon addition of MSB to a solution containing luminol and hexacyanoferrate(III), a decrease in the CL is observed. This observation forms the basis of a simple and rapid FIA-CL method for determination of MSB. A series of experiments was conducted to establish the optimum analytical variables. The parameters optimized included reagent concentrations and some physical variables, including the flow rate and the volume of sample loop.

Effect of sodium hydroxide concentration

Luminol reacts with potassium hexacyanoferrate(III) to produce light emission in basic solution. Therefore, sodium hydroxide was added in a flow line to improve the sensitivity of reaction. The concentration of sodium hydroxide versus signal/noise (S/N) ratio was studied at different

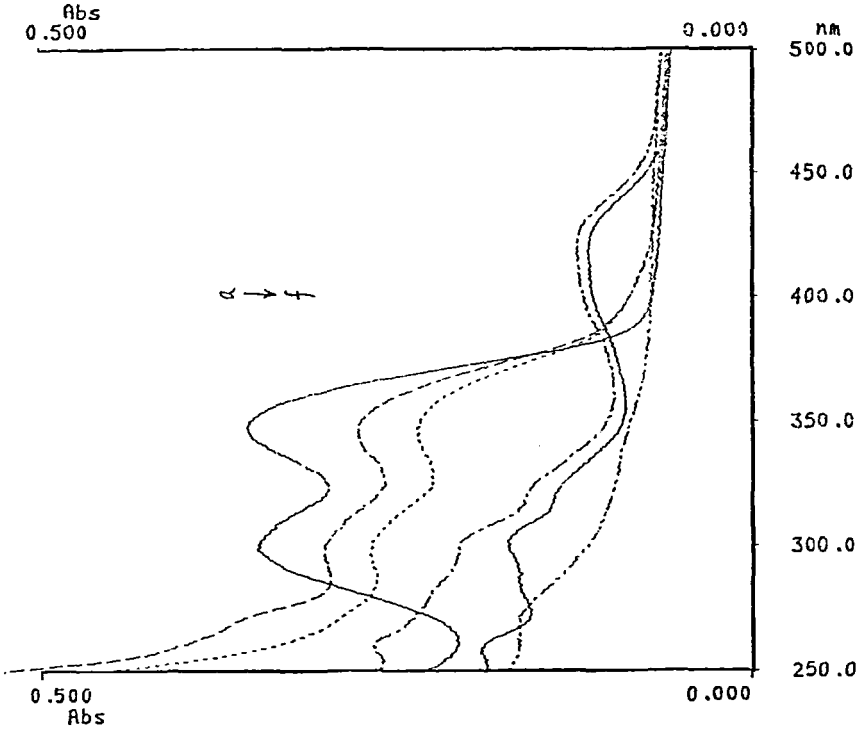

Fig.2. UV spectrum of reaction products. (a):30μM luminol + 0.2M NaOH; (b): 30μM luminol + 0.2M NaOH + 50 μM potassium hexacyanoferrate(III) + 1 μg/mL MSB; (c): 30μM luminol + 0.2M NaOH + 50 μM potassium hexacyanoferrate(III); (d): 50 μM potassium hexacyanoferrate(III) + 0.2M NaOH; (e): 50 μM potassium hexacyanoferrate(III) + 0.2M NaOH + 1 μg/mL MSB; (f): 0.2M NaOH + 1 μg/mL MSB.

concentrations from 0.1-0.6 M. The S/N ratio increases with the concentration of sodium hydroxide up to 0.4 M, thereafter remaining almost constant. Therefore, 0.4 M of sodium hydroxide was selected for the present work.

Effect of potassium hexacyanoferrate(III) concentration

The effect of sodium hydroxide concentration on the signal/noise (S/N) ratio was examined in the range from 5-200 μM. It was found that S/N ratio increases with the concentration of potassium hexacyanoferrate(III) up to 50 μM, thereafter remaining almost constant. Therefore, 50 μM of was used in the coming work.

Effect of luminol concentration

The effect of luminol concentration on the signal/noise (S/N) ratio was investigated for the range 5-100 μM. 30 μM of luminol was found to be optimum for the highest S/N ratio and hence, was selected in the subsequent experiments.

Effect of flow rate

The effect of flow rate on S/N ratio was examined in the range from 0.5 to 2 ml min^{-1} (per tube). It was found that S/N ratio increases with increase in flow rate probably because this CL reaction is a fast process. As a compromise between reagent consumption and sensitivity, 1.5 ml min^{-1} of flow rate (per tube) was recommended.

Effect of sample loop volume

It is well-known that the sample injection volume in a FIA set-up affects the signal intensity. Usually sample volume increase could lead to increase (enhancement CL) or decrease (inhibition CL) in the signal intensity. A similar

tendency was observed in this system. The examined sample volume ranges were from 25 to 200 μL. It was found that the S/N ratio increased with increase in sample volume. However, the S/N ratio increased slowly for sample volume above 70 μl. Therefore, 70 μl was considered the optimum sample injection volume in the present FI system.

Performance of the proposed method for MSB measurements

Under the selected conditions given above, the calibration graph was linear in the 0-1 μg/mL range (I = -753 [MSB] (μg/mL) +1515; r=0.99, n=8) with a detection limit (3σ) of 0.01 μg/mL. Relative standard deviations (n=11) was 0.16% for 0.4 μg/mL.

Interference study

The effect of foreign substances was tested by analyzing a standard solution of MSB (0.4μg/mL) to which increasing amounts of interfering substances were added. The tolerable concentration ratios with respect to 0.4μg/mL MSB for interference at 5% level were over 1000 for KCl, NaCl, urea; 500 for sucrose, fructose, lactose; $NaHCO_3$; 100 for Ca^{2+}, amylum; 75 for $H_2PO_4^-$; 50 for NH_4^+; 10 for Zn^{2+}; 5 for Mg^{2+}, glucose; 1 for ascorbic acid; 0.75 for Cu^{2+}, respectively.

Sample analysis

Following the procedure detailed under Section 2, the proposed method was applied to the determination of MSB in tablets and injections samples. The results are listed in Table 1, and agree well with those obtained by UV spectrophotometry[25].

Table 1 Results of the analysis of MSB in pharmaceutical preparations

Sample	Amount(mg)			Added (mg)	Found (mg)	Recovery (%)
	Label (mg)	Found(mg)±RSD% Proposed method[a]	UV method[b]			
Tablet 1	4	3.96±2.1	4.00±1.1	2.00	1.98	99.0
				4.00	4.11	102.8
				6.00	6.04	100.7
Tablet 2	4	4.00±0.7	3.94±1.5	2.00	1.93	96.4
				4.00	3.89	97.3
				6.00	6.17	102.9
Injection 1	4	3.95±1.1	3.97±2.5	2.00	1.97	98.6
				4.00	3.97	99.1
				6.00	6.11	101.8
Injection 2	4	3.99±0.8	4.01±1.6	2.00	2.05	102.5
				4.00	3.96	99.0
				6.00	5.95	99.2

[a]Average of ten measurements

[b]Average of five measurements

CONCLUSION

The proposed method promises a simple, rapid, sensitive and very cheap method for the determination of MSB, which can be applied to the analysis of pharmaceutical preparations. Determination of MSB in biological samples (such as serum and urine) by HPLC with the proposed CL detection system is now under study.

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