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# Determination of Folic Acid by Solid-Phase Extraction and Flow Injection Chemiluminescence

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Based on the chemiluminescence emitting reaction between cerium(IV) sulfate and sodium sulfite in acid medium, enhanced with folic acid. A simple, rapid, accurate and sensitive method of solid-phase extraction and flow injection chemiluminescence was developed for the determination of folic acid. The optimized experimental conditions were evaluated. Under optimum conditions, calibration curve over the range of 0.1-7.0 μg $L^{-1}$ was obtained. The detection limit of this method was 0.02 μg $L^{-1}$. The relative standard deviation was 3.9 % for 1.0 μg $L^{-1}$ folic acid. The method has been applied to the determination of studied folic acid in infant formula milk powder with satisfied result.

**Key Words: Folic acid, Flow injection, Chemiluminescence, Cerium(IV) sulfate, Sodium sulfite.**

## INTRODUCTION

Folic acid is a significant component of the haematopoietic system and is the coenzyme that controls the generation of ferrohaeme. The decrease in concentration of folic acid in human body fluids leads to several complications including gigantocytic anaemia, leucopoenia, devolution of mentality, psychosis and increasing possibility of heart attack and stroke[1]. In January 1998, the U.S. Food and Drug Administration (FDA) implemented a program to fortify all flour and cereal products with folic acid at the level of 140 μg/100 g of product[2] and the widespread use of folate-fortified dietary[3] supplements (nutraceuticals, multivitamin supplements, multivitamin/multielement supplements, *etc*.). However, folic acid deficiency remains one of the most common vitamin deficiencies worldwide.

Therefore, it is important to develop simple, sensitive and accurate methods for being able to detect folic acid. But analysis of folic acid is not easy due to its lower stability, presence in lower concentration in biological systems and complex extraction and detection techniques. Methods of analysis of folic acid are grouped into microbiological assay[4,5], chromatographic[6,7], enzyme protein binding assay[8],

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spectrophotometric and fluorescence spectrometry method[9,10]. Although microbiological assay is the most commonly used method, it is time consuming, needs great care and skill. Chromatographic methods have the advantage of separating and quantifying different forms of folic acid and its derivations and minimum interference from enzymes but involve set up cost, a complex extraction and purification procedure. Enzyme protein binding assay is much cheaper, rapider and easier but there exits considerable variation between different kits and self-life of kits is very short. The detection limit of sectrophotometric can not meet the requirement of lower concentration in biological systems and there are many disturbance factor (such as temperature, degree of acidity, solvent *etc*.) in fluorescence spectrometry. Chemiluminescence (CL) is a powerful analytical technique that has excellent sensitivity, wide linear dynamic range and requires relatively simple and inexpensive instrumentation. there were a few reports for the determination of folic acid based on chemiluminescence analysis system[11,12].

In this study, we found that a strong chemiluminescence signal was given out when a trace amount of folic acid was added to cerium(IV) sulfate and sodium sulfite mixed solution and the chemiluminescence intensity was strongly dependent on folic acid concentration. Based on this phenomenon and solid-phase extraction technique, a new, rapid, simple, sensitive and inexpensive method is proposed to determine folic acid in infant formula milk powder. It has more widely linear range and much lower detection limit. The method has been applied to the determination of studied folic acid in infant formula milk powder with a satisfied result.

## EXPERIMENTAL

The folic acid was obtained from Sigma-Aldrich (St. Louis, USA). A stock solution (50 mg $L^{-1}$) was prepared by dissolving 5.00 mg folic acid in 1 mL of 0.1 M NaOH solution and diluting to 100 mL with water. The solution was stored at 4 ºC and diluted to working solutions with water. A 0.025 mol $L^{-1}$ sodium sulfite solution was prepared by dissolving 0.1580 g sodium sulfite (Tianjin Chemical Reagent, China) in 50 mL water. A 0.010 mol $L^{-1}$ $Ce(SO_4)_2$ solution was prepared by dissolving 0.2020 g of $Ce(SO_4)_2$ (Tianjin Chemical Reagent, China) in 5.00 mL 0.5 mol $L^{-1}$ $H_2SO_4$ and then diluted with water to 50 mL. Sodium acetate, cyclohexane, methanol, HCl, $HNO_3$, $H_2SO_4$, $H_3PO_4$ and acetic acid were obtained either from Beijing Chemical Reagent Company (Beijing) or from Tianjin Chemical Reagent Company (Tianjin, China). All the above reagents were of analytical grade and used as received without further purification. Double-distilled water (referred to pure water thereafter) was used as carrier flow and for the preparation of solutions. The diluted working solutions were prepared and used freshly and daily.

**Solid-phase extraction of sample procedure:** 1 g folic acid samples diluting to 50 mL with water and filtered through a 0.45 μm membrane filter. Solid-phase extraction of folic acid samples cartridges with chromabond-SB vacuum (500 mg, 3 mL) were used as clean-up and enrichment devices.The procedure according to

the following steps: (1) **Condition:** 10 mL cyclohexane followed by 10 mL methanol and by 10 mL distilled water. (2) **Load:** 10 mL folic acid samples were applied to the cartridges. (3) **Elute:** 10 mL 0.1 mol $mL^{-1}$ sodium acetate solution (contining 10 % sodium chloride). (4) Elutropic solution were diluted for the flow injection chemiluminescence analysis.

Flow injection chemiluminescences were performed with IFFL-D flow injection chemiluminescence analysis system (Xi'an Ruike Electronic equipment Corporate, Xi'an, China). The schematic diagram of the FI-CL analyzer is shown in Fig. 1. It consisted of two peristaltic pumps (working at a constant flow rate: 30 rpm), one channels was used to carry $H_2SO_4$ and $Ce(SO_4)_2$ solution, another channels was used to carry sodium sulfite solution. Sample solutions were then injected from a sample valve. The enhanced chemiluminescence signals were produced immediately and were recorded. The flow cell was a 10 cm long spiral glass tubing (2.0 mm i.d.) and the distance between injection valve and flow cell was about 15 cm.

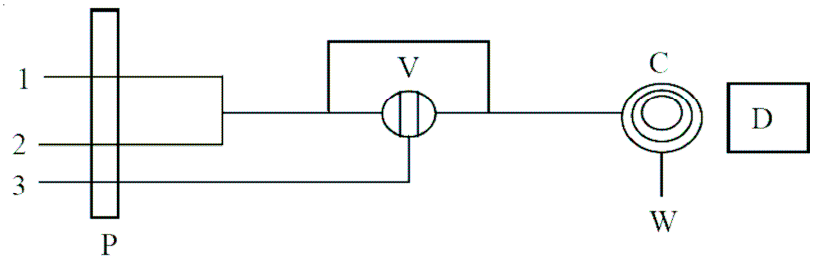


Fig. 1. Schematic of flow injection chemiluminescence analysis (1) Mixture of $H_2SO_4$ and $Ce(SO_4)_2$; (2) sodium sulfite solution; (3) folic acid solution; (C) Flow cell; (V) Injection valve; (W) Waste solution; (D) Detector.

## RESULTS AND DISCUSSION

**Optimization of solid-phase extraction conditions:** Solid-phase extraction constitutes a good alternative to other commonly used extraction methods as sampling can be done rapidly and directly[13]. In the solid-phase extraction of folic acid in milk powder, many factors had to be taken into account. Thus, a strategy was applied in which several factors were studied and optimized sequentially. Firstly, for the selection of extraction solvent preliminary experiments were taken into consideration. Several of medium to extracting agents such as phosphate solution, sodium acetate solution, sodium chloride solution, methanol and dichloromethane were used and different binary solvent mixtures were evaluated. The solvent, 0.1 mol $mL^{-1}$ sodium acetate solution yielded higher extraction efficiency for most of the target compounds. 0.1 mol $mL^{-1}$ sodium acetate solution was selected as the most appropriated solvent. Secondly, the sodium acetate solution volume was evaluated from 7.0 to 12.0 mL . According to the results obtained, the responses of all analytes gradually augmented when the sodium acetate solution increased from 7.0 to 12.0 mL, but the differences

found between 10 and 12.0 mL were insignificant. From the results it was evident that 10 mL of 0.1 mol $mL^{-1}$ sodium acetate solution allowed an efficient extraction volume of the analytes.

**Optimization of chemiluminescence conditions**

**Effect of flow rate on chemiluminescence intensity:** The chemiluminescence intensity increased with increasing the flow rate, but if the flow rate was too slow or too fast, a suitable chemiluminescence intensity could not be obtained. A flow rate of 30 rpm for all solutions seemed to give the best results and thus was employed in present measurements.

**Effect of carrier flow:** A number of carrier flows were tested at the selection conditions: ([FA] = 1.0 μg $L^{-1}$; [$Ce(SO_4)_2$] = $0.6 \times 10^{-3}$ mol $L^{-1}$; [sodium sulfite] = $1.0 \times 10^{-3}$ mol $L^{-1}$), those flows included HCl; $HNO_3$; $H_2SO_4$; $H_3PO_4$ and acetic acid solutions. The experiment indicated that the chemiluminescence emission intensities was the most sensitive in $H_2SO_4$ solution. The influence of $H_2SO_4$ concentration on chemiluminescence emission intensities was investigated further. To our surprise, the use of $3.0 \times 10^{-3}$ mol $L^{-1}$ of $H_2SO_4$ solution as a carrier flow gave the best result; thus, we chose $3.0 \times 10^{-3}$ mol $L^{-1}$ of $H_2SO_4$ solution as the carrier flow.

**Influence of sodium sulfite concentration on chemiluminescence intensities:** The influence of sodium sulfite concentration on chemiluminescence emission intensities was studied; it was found that chemiluminescence intensities increased with the increase in sodium sulfite concentration (Fig. 2). On the other hand, the base signal of chemiluminescence generated by the reaction between cerium(IV) and sodium sulfite also increased with the concentration of sodium sulfite. In turn, the signal-to-noise ratio (S/N) increased as well. After an analysis of S/N ratio of the baseline and the sensitivity of the system, the best concentration of sodium sulfite was $1.0 \times 10^{-3}$ mol $L^{-1}$.

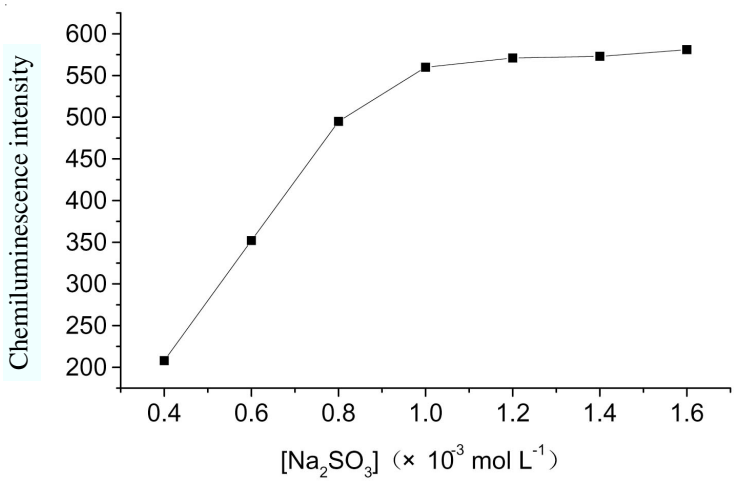


Fig. 2. Effect of sodium sulfite concentration on the chemiluminescence response

**Effect of cerium(IV) concentration on chemiluminescence intensities:** Fig. 3 shows the chemiluminescence intensities as a function of cerium(IV). Obviously, maximum chemiluminescence intensity was obtained when cerium(IV) was at 0.6 × $10^{-3}$ mol $L^{-1}$. The optimum concentration of cerium(IV) was therefore chosen as 0.6 × $10^{-3}$ mol $L^{-1}$.

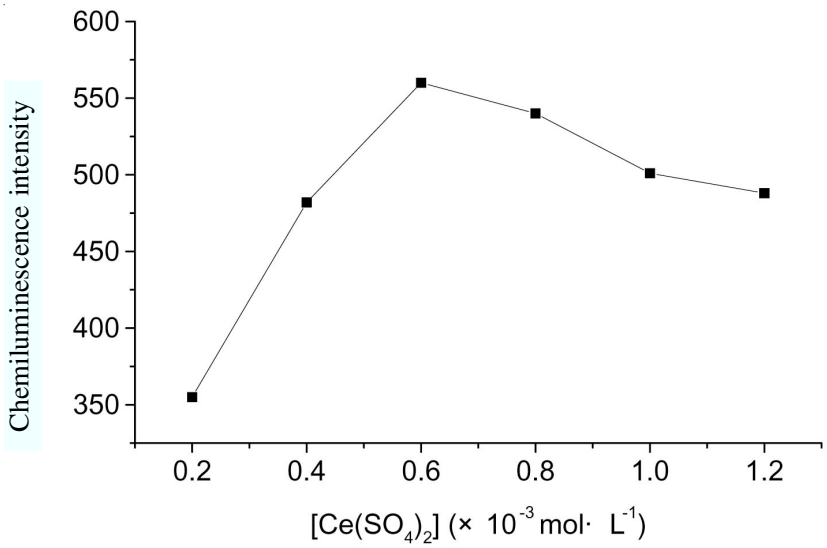


Fig. 3. Effect of cerium(IV) concentration on the chemiluminescence response

**Performance of the system for folic acid measurements:** Under the optimal conditions described above, the linear detection range was from 0.1 to 7.0 μg $L^{-1}$. A regression equation was obtained as: Intensity = 35.4 + 308.9c (c: μg $L^{-1}$, r = 0.9991). The detection limit (3σ) for the regression equation was 0.02 g $L^{-1}$ and the relative standard deviation (RSD, n = 11) was 3.9 % for 1.0 μg $L^{-1}$ folic acid.

**Study of interferences:** The influences of some common inorganic ions and relevant organic compounds on chemiluminescence intensities were investigated for measuring 1.0 μg $L^{-1}$ folic acid. The tolerable concentration ratios with respect to 1.0 μg $L^{-1}$ folic acid standard solution for interference at less than 5 % level were over: 1000 for $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Fe^{3+}$, $Mg^{2+}$, $Al^{3+}$, $Zn^{2+}$, $Cu^{2+}$; 500 for sodium tartrate, citrate, glucose, fructose; 50 for L-histidine acid, L-glutamic acid, glycine, glycine and urea, respectively.

**Sample analysis:** After the solid-phase extraction clean-up procedure as described in the experimental section, Then the proposed method was applied to the determination of folic acid in 5 infant formula milk powder. The recovery experiment of adding standard had been done at the same time. The determination results were shown in Table-1 indicated that the folic acid content in all the five samples tested was over the labeled value.

TABLE-1
RESULTS OF FOLIC ACID IN MILK POWDER

| Sample | Labeled (μg 100 $g^{-1}$) | Found (μg 100 $g^{-1}$) | Added (μg 100 $g^{-1}$) | Total found (μg 100 $g^{-1}$) | Recovery (%) |
|---|---|---|---|---|---|
| 1 | 30 | 38.7 | 50.0 | 86.2 | 97.2 |
| 2 | 40 | 49.3 | 50.0 | 95.1 | 95.8 |
| 3 | 50 | 60.5 | 50.0 | 115.3 | 104.3 |
| 4 | 55 | 62.1 | 50.0 | 108.4 | 96.7 |
| 5 | 70 | 75.7 | 50.0 | 120.3 | 95.7 |

## Conclusion

In this paper, a flow-injection chemiluminescence system combined with solid-phase extraction was proposed for determination of folic acid at μg $L^{-1}$ level. It showed that flow-injection with solid-phase extraction approach resulted in significantly enhanced sensitivity and selectivity of chemiluminescence method. It provides an alternative method to determine folic acid in milk powder, which implies the application to nutraceuticals, multivitamin supplements, multivitamin/ multielement supplements also.

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