JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Flow Injection Chemiluminescence Determination of Sudan I in Hot Chilli Sauce

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A chemiluminescence method based on the luminol−H_2O_2 system with flow injection technology was proposed for the determination of sudan I in hot chilli sauce. It was found that sudan I could enhance chemiluminescence intensity generated from the luminol−H_2O_2 system. The increment of chemiluminescence intensity was proportional to the concentration of sudan I, giving a calibration graph linear over the concentration from 10 pg mL^{-1} to 7 ng mL^{-1} ($R^2 = 0.9980$) with the detection limit of 3 pg mL^{-1} (3σ) and the quantification limit of 7.5 pg mL^{-1}. At a flow rate of 2.0 mL min^{-1}, one analysis cycle, including sampling and washing, could be accomplished in 60 s with a relative standard deviation of <5.0%. The method has been applied successfully to the determination of sudan I in Pixian douban, Golden Mark guilin chilli sauce, and Golden Mark satay sauce, and the recovery was 90.6−110.0%.

KEYWORDS: Sudan I; chemiluminescence; luminol; flow injection

INTRODUCTION

Sudan I is a synthetic azoic dye (**Figure 1**) that is typically used in many industrial applications including solvents, oils, fats, waxes, plastics, printing inks, and floor polishes (*1*, *2*). The main reason for the widespread usage is its colorfastness and low price. However, azo-colorants are biologically active through their metabolites (*3*, *4*) and have been associated with increased occurrence of bladder cancer in textile and leather dyers, painters, and hairdressers (*5*, *6*). Due to their potential carcinogenicity, many countries have banned the use of most azo dyes at any level in products for human consumption (*5*). It is well-known that sudans (I−IV) have been classified by the International Agency for Research on Cancer (IARC) as category 3 carcinogens because they can induce some forms of liver and bladder cancer in animals (*7*), and the use of sudan I in foodstuff is forbidden by a global food regulation act (*8*). For this reason, an accurate and reliable method for the determination of sudan I in foodstuff is required for the assurance of consumer health.

Among the methods available, high-performance liquid chromatography (HPLC) coupled with different types of detector, involving MS (*9*−*11*), UV (*12*−*15*), and chemiluminescence (CL) (*16*), has been widely used for the determination of sudan I. Other analytical procedures, including solid-phase extraction (*17*), isotope dilution combined with MS (*18*), and solid-phase spectrophotometry (*19*), have been reported. HPLC-MS/MS has also been used to detect sudan I in trace amounts in foods (*20*).

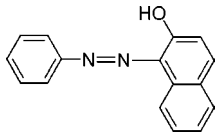

Figure 1. Structure of sudan I.

However, no report has been found on a CL method for the direct assay of sudan I to date.

CL has attracted increasing attention in various fields owing to its high sensitivity, wide linear range, and simple instrumentation (*21*−*25*). In this paper, it was found that sudan I could enhance the CL intensity generated from the luminol−H_2O_2 oxidation reaction in alkaline medium. The enhancement of CL intensity was proportional to the concentration of sudan I ranging from 10 pg mL^{-1} to 7 ng mL^{-1} ($R^2 = 0.9980$) with the detection limit (LOD) of 3 pg mL^{-1} (3σ) and the quantification limit (LOQ) of 7.5 pg mL^{-1}. Moreover, at a flow rate of 2.0 mL min^{-1}, the procedure could be accomplished in 1 min, including sampling and washing, offering the sampling efficiency of 60 h^{-1} with a relative standard deviation of <5.0%. The proposed method has been applied successfully to the determination of sudan I in hot chilli sauce, and the recovery was 90.6−110.0%.

EXPERIMENTAL PROCEDURES

Reagents. All chemicals used were of analytical reagent grade. Water purified in a Milli-Q system (Millipore, Bedford, MA) was used throughout. Luminol (Fluka, Biochemika) was obtained from Xi'an Medicine Purchasing and Supply Station, China. Hydrogen peroxide was purchased from Xi'an Chemical Reagent Plant. Standard solutions of sudans I−IV and samples of sudan I were supplied by the Shaanxi Entry−Exit Inspection and Quarantine Bureau.

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10.1021/jf063332h CCC: $37.00
Published on Web 01/06/2007

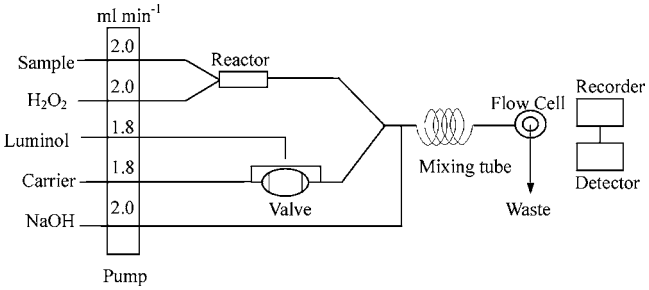

Figure 2. Schematic diagram of the present flow injection CL system for sudan I determination.

Stock solutions of sudan I (100 μg mL^{-1}) and sudan II (512 μg mL^{-1}) were prepared in acetonitrile and stored at 4 °C, whereas stock solutions of sudan III (506 μg mL^{-1}) and sudan IV (504 μg mL^{-1}) were prepared in chloroform and stored at 4 °C. Working standard solutions of sudan I were prepared daily from the above stock solutions as required. Luminol (2.5×10^{-2} mol L^{-1}) was prepared by dissolving 4.4 g of luminol in 1 L of 0.1 mol L^{-1} NaOH solution. A 0.1 mol L^{-1} stock standard solution of hydrogen peroxide was prepared by dissolving the solution in distilled water.

Apparatus. A schematic diagram of the CL flow injection analysis system is shown in **Figure 2**. A peristaltic pump was utilized to deliver all flow streams. PTFE tubing (1.0 mm i.d.) was used as connection material in the flow system, and the whole system was pumped. A six-way valve with a loop of 100 μL was employed for sampling. The flow cell was made by coiling 15 cm of colorless glass tube (i.d. = 2 mm) into a spiral disk shape with a diameter of 2 cm and placed close to the photomultiplier tube (PMT) (Hamamatsu, model IP28). The CL signal produced in the flow cell was detected without wavelength discrimination, and the negative high voltage (−730 V) was supplied to the PMT by a luminosity meter (Xi'an Remax Electronic Science-Technology Co. Ltd., model GD-1) connected with a recorder (Shanghai Dahua Instrument and Meter Plant, model XWT-206).

Procedures. As shown in **Figure 2**, flow lines were inserted into the sample, H_2O_2, luminol, carrier (pure water), and sodium hydroxide solutions, respectively. The pump was started at a constant speed of 2.0 mL min^{-1} to wash the whole system until a stable baseline was recorded. Then 100 μL of luminol solution was injected into the carrier stream by injection valve, merged with the mixed solution stream of sudan I and H_2O_2. The mixed solution in an alkaline medium was delivered into the CL cell, producing CL emission, detected by the PMT and luminometer. The concentration of the sample was quantified by the increment of CL intensity, $\Delta I = I_s - I_0$, where I_s and I_0 are CL signals in the presence and in the absence of sudan I, respectively.

Sample Preparation. The proposed procedure for the determination of sudan I was applied to hot chilli sauce. Contaminated hot chilli sauces were ground to a fine powder using a Multifunctional Food Cooking Unit (Shangdong Jiu Yang Small Electrical Appliance Co. Ltd., model JYL-350); about 3 g was weighed and dissolved ultrasonically for 30 min in 1:1 (v/v) acetonitrile (*26*) and water in a PTFE digestion can, incubated, and centrifuged for 10 min, and then the upper clear solution was determined.

RESULTS AND DISCUSSION

CL Intensity−Time Profile of Sudan I in Luminol−Hydrogen Peroxide System. The intensity−time profile of the reaction of luminol and hydrogen peroxide was tested using luminol 2×10^{-6} mol L^{-1} in 0.025 mol L^{-1} sodium hydroxide in the flow system. As **Figure 3** shows, CL intensity reached the maximum at 15 s after the reagents were mixed and vanished within 60 s thereafter. It can be seen that the CL evidently increased in the presence of sudan I and varied with the concentration of sudan I.

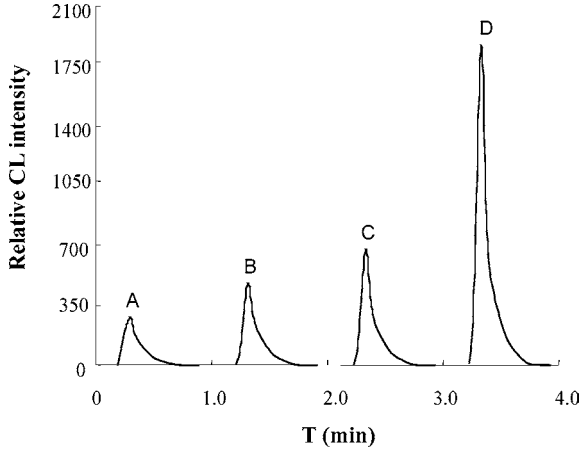

Figure 3. CL intensity–time profile of sudan I in luminol–hydrogen peroxide system. Peaks: A, CL intensity in the absence of sudan I; B, CL intensity in the presence of 70 pg mL^{-1} sudan I; C, CL intensity in the presence of 700 pg mL^{-1} sudan I; D, CL intensity in the presence of 7000 pg mL^{-1} sudan I.

Effect of Luminol, Hydrogen Peroxide, and Sodium Hydroxide Concentration. The effect of luminol and hydrogen peroxide concentration on the CL intensity was investigated over the ranges of 1.0×10^{-10} to 1.0×10^{-5} mol L^{-1} and 1.0×10^{-7} to 1.0×10^{-3} mol L^{-1}, respectively. The maximum relative CL intensity could be obtained when using a concentration of 2×10^{-6} mol L^{-1} luminol; concentrations under or above 2×10^{-6} mol L^{-1} luminol caused a sharp decrease in the CL intensity. Therefore, 2×10^{-6} mol L^{-1} of luminol was selected for the present work. With regard to the concentration of hydrogen peroxide, 1.0×10^{-4} mol L^{-1} hydrogen peroxide gave the maximum CL intensity. Hence, a concentration of 1.0×10^{-4} mol L^{-1} was selected for subsequent work.

Owing to the nature of luminol CL reaction, which is more favored in alkaline medium, sodium hydroxide was added in the flow to improve the sensitivity of the system. The effect of the sodium hydroxide concentration was studied over the range from 0.01 to 0.025 mol L^{-1}. The CL intensity increased when the sodium hydroxide concentration was increased up to 0.025 mol L^{-1}, but decreased at a higher concentration. Therefore, 0.025 mol L^{-1} sodium hydroxide was used in all subsequent experiments.

Effect of Flow Rate and Length of Mixing Tube. The effect of flow rate on CL intensity was examined in the range from 0.5 to 5 mL min^{-1}. It was found that the CL intensity increased with increase in flow rate, probably because this CL reaction was a fast process. As a compromise between reagent consumption and sensitivity, a 2.0 mL min^{-1} flow rate was recommended.

The length of the mixing tubing was also adjusted to yield maximum light emission in the cell. It was found that an 11.0 cm mixing tube afforded the best results with good sensitivity and reproducibility.

Performance of Proposed Method for Sudan I Measurement. Under the optimum conditions described, the calibration graph of CL increment (ΔI) versus sudan I concentration was linear in the range of 10−7000 pg mL^{-1}, given the regression equation $\Delta I_{CL} = 0.2008 C_{\text{sudan I}} + 239.3$ ($R^2 = 0.9980$, $n = 7$), with a detection limit of 3 pg mL^{-1} (3σ). A complete analysis, including sampling and washing, could be performed in 1 min with relative standard deviations (RSD) of 4.79, 1.70, and 1.07% for 10, 700, and 7000 pg mL^{-1}, respectively.

Table 1. Calibration Curves, Detection Limits (LOD), and Quantification Limits (LOQ) of Sudan Dyes[a]

sudan	linear range, ng mL^{-1}	LOD, ng mL^{-1}	LOQ, ng mL^{-1}	regression eq	R^2 [b]
I	0.01–7	0.003	0.0075	$\Delta I_{CL} = 0.20C_{sudan\ I} + 239.3$	0.9980
II	1–500	0.3	0.75	$\Delta I_{CL} = 11.36C_{sudan\ II} + 205.5$	0.9994
III	10–1000	3.0	7.5	$\Delta I_{CL} = 0.63C_{sudan\ III} + 403.0$	0.9996
IV	10–500	3.0	7.5	$\Delta I_{CL} = 0.74C_{sudan\ IV} + 535.9$	0.9880

[a] The relative standard deviations of all data presented were <5.0% ($n = 7$). [b] R^2, correlation coefficient.

Table 2. Results of Determination of Sudan I in Pixian Douban, Golden Mark Guilin Chilli Sauce, and Golden Mark Satay Sauce[a]

					amount of sudan I in sample, μg g^{-1}	
sample[b]	added, pg mL^{-1}	found, pg mL^{-1}	RSD %	recovery %	by the proposed method	by HPLC[c]
1	0	8.3	2.92	90.6		
	10	17.3	2.83		4.72	
2	0	8.0	2.66	110.0		
	10	19.0	1.09			
3	0	15.1	2.88	94.6		
	20	34.1	2.11		1.41	
4	0	3.3	2.19	100.0		
	10	13.3	1.39			
5	0	8.6	2.28	94.6		
	10	18.1	2.07		1.34	1.33
6	0	2.6	3.39	98.1		
	10	12.5	1.89			

[a] The average of seven determinations. [b] Samples 1 and 2, Pixian douban; samples 3 and 4, Golden Mark guilin chilli sauce; samples 5 and 6, Golden Mark satay sauce. [c] Undetected for sudans II, III, and IV.

Linearity, Limit of Detection, and Limit of Quantification of Sudans I−IV. Under the optimum conditions described above, the results obtained with the proposed method of determination of sudans I−IV are listed in **Table 1**. It could be found that the concentrations of sudans I−IV versus relative CL intensity showed a linear relationship ($R^2 \geq 0.9880$) in the ranges of 0.01−7, 1−500, 10−1000, and 10−500 ng mL^{-1}. Detection limits of sudans I−IV were 0.003, 0.3, 3, and 3 ng mL^{-1} and quantification limits were 0.0075, 0.75, 7.5, and 7.5 ng mL^{-1}, respectively. It could be also seen that sudan I had the widest linear range and the lowest detection limit in comparison with sudans II, III, and IV.

Interference Studies. The interference of foreign substances was investigated by analyzing a standard solution of sudan I (50 pg mL^{-1}) to which increasing amounts of interfering analyte were added. The tolerable concentrations with respect to 50 pg mL^{-1} sudan I for interference at a 5% level were over 30 μg mL^{-1} for glutin, barbiturate, oxalic acid, urea, and acetone, 7.0 μg mL^{-1} for methanol and ethanol, 1.2 μg mL^{-1} for albumin, 1.0 μg mL^{-1} for Cl^-, NO_3^-, Ac^-, I^-, SO_4^{2-}, PO_4^{3-}, $Cr_2O_7^{2-}$, borate, oxalate, tartrate, citrate, and malic acid, 0.7 μg mL^{-1} for globulin, 0.5 μg mL^{-1} for NH_4^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, L-threonine, and myoglobin, 0.3 μg mL^{-1} for uric acid, 0.3 μg mL^{-1} for L-leucine, 0.2 μg mL^{-1} for L-hydrochloric acid lysine, 0.1 μg mL^{-1} for L-cysteine, 10 ng mL^{-1} for L-histidine, 0.5 ng mL^{-1} for Co^{2+}, Fe^{3+}, Fe^{2+}, and Mn^{2+}, 0.5 ng mL^{-1} for sudan II, and 5 ng mL^{-1} for sudans III and IV.

Application: Determination of Sudan I in Contaminated Hot Chilli Sauce. The sample of hot chilli sauce, which was supplied by the Shananxi Entry−Exit Inspection and Quarantine Bureau, has been confirmed to contain sudan I. The proposed method was applied to the determination of sudan I in Pixian douban, Golden Mark guilin chilli sauce, and Golden Mark satay sauce. Two samples were prepared as described under Sample Preparation; one of the samples was added to a 5 mL standard solution (1 μg mL^{-1}), respectively. The samples were determined directly, and the results are listed in **Table 2**.

Table 3. Results of Determination of Sudan I in Spiked Samples[a]

sample	added, pg mL^{-1}	found, pg mL^{-1}	RSD %	recovery %	content in sample, μg, by the proposed method/spiked
1	0	5.4	1.63	96.3	0.48/0.5
	10	15.0	1.24		
2	0	12.0	2.38	91.3	0.46/0.5
	10	21.1	2.26		
3	0	26.2	1.87	109.8	0.55/0.5
	10	37.2	2.90		

[a] The average of seven determinations. [b] Sample 1, Golden Mark satay sauce; sample 2, Golden Mark guilin chilli sauce; sample 3, Pixian douban.

To validate the proposed method to detect sudan I specifically, known quantities of sudans I, II, III, and IV (0.5 μg) were spiked into the samples of Pixian douban, Golden Mark guilin chilli sauce, and Golden Mark satay sauce and determined. The contents of sudan I in the spiked samples were quantified according to the standard addition method, and the results are listed in **Table 3** with recoveries ranging from 91.3 to 109.8% and RSDs of <3.0%.

A sensitive and simple CL method has been proposed for the determination of sudan I in hot chilli sauces. Combined with the flow injection system, the enhanced luminol−H_2O_2 CL intensity was utilized for the determination of sudan I. The proposed method has prominent advantages including instrumental simplicity, reduced reagent consumption, improved sensitivity, analytical efficiency, and an easy handling procedure as well. The present method offers the promise for routine quality control of pharmaceuticals and determination of sudan I in food analysis.

LITERATURE CITED

(1) Habibi, M. H.; Hassanzadeh, A.; Mahdavi, S. The effect of operational parameters on the photocatalytic degradation of three textile azo dyes in aqueous TiO_2 suspensions. *J. Photochem. Photobiol. A* **2005**, *172*, 89−96.

(2) Nohynek, G. J.; Fautz, R.; Benech-Kieffer, F.; Toutain, H. Toxicity and human health risk of hair dyes. *Food Chem. Toxicol.* **2004**, *42*, 517−543.

(3) Golka, K.; Kopps, S.; Myslak, Z. W. Carcinogenicity of azo colorants: influence of solubility and bioavailability. *Toxicol. Lett.* **2004**, *151*, 203−210.

(4) Pinheiro, H. M.; Touraud, E.; Thomas, O. Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters. *Dyes Pigments* **2004**, *61*, 121−139.

(5) Ahlstrom, L. H.; Sparr Eskilsson, C.; Bjorklund, E. Determination of banned azo dyes in consumer goods. *Trends Anal. Chem.* **2005**, *24*, 49−56.

(6) Nohynek, G. J.; Fautz, R.; Benech-Kieffer, F.; Toutain, H. Toxicity and human health risk of hair dyes. *Food Chem. Toxicol.* **2004**, *42*, 517−543.

(7) International Agency for Research on Cancer (IARC). *Some Aromatic Azo Compounds, IARC, Sudan I*; IARC Monograph; IARC: Lyon, France, 1975; Vol. 8, pp 225−231.

(8) Di Donna, L.; Maiuolo, L.; Mazzotti, F.; De Luca, D.; Sindona, G. Assay of sudan I contamination of foodstuff by atmospheric pressure chemical ionization tandem mass spectrometry and isotope dilution. *Anal. Chem.* **2004**, *76*, 5104−5108.

(9) Calbiani, F.; Careri, M.; Elviri, L.; Mangia, A.; Pistarà, L.; Zagnoni, I. Development and in-house validation of a liquid chromatography−electrospray−tandem mass spectrometry method for the simultaneous determination of sudan I, sudan II, sudan III and sudan IV in hot chilli products. *J. Chromatogr. A* **2004**, *1024*, 123−130.

(10) Calbiani, F.; Careri, M.; Elviri, L.; Mangia, A.; Zagnoni, I. Accurate mass measurements for the confirmation of sudan azo-dyes in hot chilli products by capillary liquid chromatography−electrospray tandem quadrupole orthogonal-acceleration time of flight mass spectrometry. *J. Chromatogr. A* **2004**, *1058*, 127−135.

(11) Ma, M.; Luo, X.; Chen, B.; Su, S.; Yao, S. Simultaneous determination of water-soluble and fat-soluble synthetic colorants in foodstuff by high-performance liquid chromatography−diode array detection-electrospray mass spectrometry. *J. Chromatogr. A* **2006**, *1103*, 170−176.

(12) Zhang, Y. P.; Zhang, Y. J.; Gong, W. J.; Gopalan, A. I.; Lee, K. P. Rapid separation of sudan dyes by reverse-phase high performance liquid chromatography through statistically designed experiments. *J. Chromatogr. A* **2005**, *1098*, 183−187.

(13) Mazzetti, M.; Fascioli, R.; Mazzoncini, I.; Spinelli, G.; Morelli, I.; Bertoli, A. Determination of 1-phenylazo-2-naphthol (sudan I) in chilli powder and in chilli-containing food products by GPC clean-up and HPLC with LC/MS confirmation. *Food Addit. Contam.* **2004**, *21*, 935−941.

(14) Daood, H. G.; Biacs, P. A. Simultaneous determination of sudan dyes and carotenoids in red pepper and tomato products by HPLC. *J. Chromatogr. Sci.* **2005**, *43*, 461−465.

(15) Cornet, V.; Govaert, Y.; Moens, G.; Van Loco, J.; Degroodt, J. M. Development of a fast analytical method for the determination of sudan dyes in chili- and curry-containing foodstuffs by high performance high performance liquid chromatography-photodiode array detection. *J. Agric. Food Chem.* **2006**, *54*, 639−644.

(16) Zhang, Y.; Zhang, Z.; Sun, Y. Development and optimization of an analytical method for the determination of sudan dyes in hot chilli pepper by high-performance liquid chromatography with on-line electrogenerated BrO^--luminol chemiluminescence detection, *J. Chromatogr. A* **2006**, *1129*, 34−40.

(17) Puoci, F.; Garreffa, C.; Iemma, F.; Muzzalupo, R.; Spizzirri, U. G.; Picci, N. Molecularly imprinted solid phase extraction for detection of sudan I in food matrices. *Food Chem.* **2005**, *93*, 349−353.

(18) Di Donna, L.; Maiuolo, L.; Mazzotti, F.; De Luca, D.; Sindona, G. Assay of sudan I contamination of foodstuff by atmospheric pressure chemical ionization tandem mass spectrometry and isotope dilution. *Anal. Chem.* **2004**, *76*, 5104−5108.

(19) Capitàn, F.; Capitàn-Vallvey, L. F.; Fernàdez, M. D.; De Orbe, I.; Avidad, R. Determination of colorant matters in foods by solid-phase spectrophotometry. *Anal. Chim. Acta* **1996**, *331*, 141−148.

(20) Tateo, F.; Bononi, M. Fast determination of sudan I by HPLC/APCI-MS in hot chilli, spices, and oven-baked foods. *J. Agric. Food Chem.* **2004**, *52*, 655−658.

(21) Roda, A.; Pazzagli, M.; Kricka, L. J. *Bioluminescence and Chemiluminescence Perspectives for the 21st Century*; Wiley: Chichester, U.K., 1999.

(22) Roda, A.; Guarigli, M.; Michelini, E.; Mirasoli, M.; Pasini, P. Analytical bioluminescence and chemiluminescence. *Anal. Chem.* **2003**, *75*, 463A−470A.

(23) Song, Z. H.; Wang, L. Chemiluminescence investigation of detection of rutin in medicine and human urine using controlled-reagent-release technology. *J. Agric. Food Chem.* **2001**, *49*, 5697−5701.

(24) Song, Z. H.; Hou, S. Sub-picogram determination of vitamin B_{12} in pharmaceuticals and human serum using flow injection with chemiluminescence detection. *Anal. Chim. Acta* **2003**, *488*, 71−79.

(25) Song, Z. H.; Hou, S.; Zhang, N. A new green analytical procedure for monitoring sub-nanogram amounts of chlorpyrifos on fruits using flow injection chemiluminescence with immobilized reagents. *J. Agric. Food Chem.* **2002**, *50*, 4468−4474.

(26) GB/T 19681-2005, The method for the determination of sudan dyes in foods-high performance liquid chromatography. *Meat Hygiene*, **2005**, *8*.

Received for review November 17, 2006. Revised manuscript received November 29, 2006. Accepted November 29, 2006. We gratefully acknowledge financial support from the Shaanxi Province Nature Science Foundation and Ministry of Education, China, Grants 2006B05 and 04Jk103.

JF063332H