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Sensitive fluorimetric determination of formaldehyde by the co-quenching effect of formaldehyde and sulfite on the fluorescence of tetra-substituted amino aluminium phthalocyanine

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A novel and sensitive fluorimetric method was developed for the determination of formaldehyde based on the co-quenching effect of formaldehyde and sulfite on the fluorescence of tetra-substituted amino aluminium phthalocyanine. Formaldehyde in the concentration range 0.040–1.19 μg ml^{-1} can be determined with a limit of detection of 7.5 ng ml^{-1}. The relative standard deviation for nine replicate measurements of 80.0 ng ml^{-1} formaldehyde is 1.8%. The method was applied to the analysis of real samples with satisfactory results.

1. Introduction

Formaldehyde is the most abundant gas-phase carbonyl compound in the atmosphere. It originates from both combustion sources and atmospheric oxidation of hydrocarbons. Formaldehyde has a strong influence on the global mixing rate of ozone and OH· radicals, which governs the oxidizing capacity of the troposphere.[1] The historic record of the oxidizing capacity of the atmosphere can be gleaned from an analysis of polar ice core composition. It is presently possible to measure the methane trapped therein; an estimation of the prevalent OH· radical concentration can be made if the formaldehyde concentration can also be measured.[2] On the other hand, the effect of formaldehyde on people exposed to this compound is well known, thus, determination of formaldehyde is also needed in industrial hygiene applications. Therefore, there is a strong demand for rapid and sensitive techniques for the measurement of formaldehyde.

Various methods are available for the determination of formaldehyde. Among them, spectrophotometry is most widely used. Many spectrophotometric methods require a chemical reaction of formaldehyde with various reagents such as pararosaniline and phloroglucinol to form colored compounds, which can be observed spectrophotometrically.[3–6] Several papers have been published for the determination of formaldehyde by kinetic spectrophotometric methods.[7,8] However, some of these methods are not sensitive enough and are subject to numerous interferences from phenols, alcohols and cyclohexanone. Chromatographic methods including GC and HPLC have been reported for the determination of formaldehyde based on pre- or post-column derivatization reactions.[9–17] In particular, liquid chromatography of the dinitrophenylhydrazone derivative has been shown to provide adequate sensitivity.[9–13] However, these methods require a fairly elaborate analytical procedure and a long measuring time per sample. Because of their intrinsic sensitivity, luminescence methods are particularly attractive for trace measurements. Chemiluminescence (CL) methods for the determination of formaldehyde, based on the reaction of gallic acid, alkaline H_2O_2 and HCHO,[18] or converting formaldehyde into a fluorescent derivative through a reaction with 4-amino-3-pent-3-en-2-one and then determining the derivatized HCHO by using a bis(2,4,6-trichlorophenyl)oxalate flow CL system,[19] or inhibition by formaldehyde reaction of lucigenin–ClO–H_2O_2,[20] have been reported. Polarographic or voltammetric methods have also been reported.[21–23] The determination of low levels of formaldehyde in drinking water has been reported, based on preconcentration with poly(allylamine) beads and analysis by a flow-injection fluorescence detection system incorporating immobilized formaldehyde dehydrogenase.[24]

There are many fluorescence methods used to determine formaldehyde. A more widely used fluorimetric method utilizes the Hantzsch reaction, which involves the cyclization of an amine, an aldehyde and a β-diketone to form a dihydropyridine derivative.[25] Nash[26] first introduced the use of pentane-2, 4-dione (PD) for analytical purposes in a photometric version. Later, Belman[27] discovered that a much more sensitive measurement can be made by using fluorimetry instead of colorimetry without any other changes. Nowadays, PD, cyclohexane-1,3-dione or dimedone are often used[25,28] to determine formaldehyde based on this chemistry, and have been coupled with HPLC.[17,29,30] Houdier and co-workers[31,32] have recently reported new fluorescent probes which show adequate sensitivity for the detection of formaldehyde. A fairly sensitive fluorimetric method, based on the reaction of formaldehyde with 3,4-diaminoanisole to form a fluorescent Schiff base, has also been reported, but the method needs a refluxing process, which is fairly tedious.[33]

In this work, we report a method based on the co-quenching effect of formaldehyde and sulfite on the fluorescence of tetra-substituted amino aluminium phthalocyanine (TAAlPc). The method is rapid, simple, sensitive (detection limit 7.5 ng ml^{-1}) and of good selectivity. Moreover, the interference from background fluorescence and scattered light from the matrix can

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be greatly diminished since both the excitation and emission of TAAlPc are located in the red-region. To our knowledge, similar investigations have not been reported previously.

2. Experimental

2.1 Apparatus

A Hitachi Model 650-10S fluorescence spectrophotometer equipped with a xenon lamp, dual monochromators, a 1 × 1 cm cell and a functional recorder was used. The slit-width for both the excitation and emission monochromators was set at 5 nm.

2.2 Reagents

The fluorescent dye 4,4′,4″,4‴-tetrasubstituted amino aluminium phthalocyanine (TAAlPc) was synthesized and purified according to the method reported previously.[34] A stock solution of TAAlPc (1.0×10^{-3} mol l^{-1}) was prepared by dissolving solid TAAlPc in redistilled dimethylformamide. Formaldehyde stock solution (2.00 mg ml^{-1}) was prepared by diluting an appropriate amount of commercial aqueous formaldehyde solution (36%, Merck) with water and was standardized by the iodimetric method. Formaldehyde standard solutions were prepared daily from the stock solution by appropriate dilution with water. A sodium sulfite solution (1%) was prepared daily by dissolving 1.000 g of solid Na_2SO_3 in distilled de-ionized water. A HCl solution (1 mol l^{-1}) was prepared by diluting 8.33 ml of 12 mol l^{-1} HCl to 100 ml with water. All chemicals were of analytical-reagent grade and were used without further purification. Distilled de-ionized water was used throughout.

2.3 Sampling process

2.3.1 Air sample. Since formaldehyde is a readily soluble gas, a traditional bubbler was used to sample formaldehyde in the air of an animal specimens room. About 20 ml of distilled de-ionized water were placed in each of two flasks, and the bubbling rate was maintained at 0.35 l min^{-1}. After a fixed period of time, the contents of the two flasks were transferred into a 50 ml flask and diluted to the mark.

2.3.2 Treatment of phenol–formaldehyde resin sample. A 0.5000 g amount of the resin was accurately weighed and suspended in water. The free formaldehyde was separated by steam distillation, collecting 250 ml of the distillate.

The formaldehyde contents of the two above-mentioned samples were determined by the recommended procedure.

2.4 Procedure

To a 10 ml calibrated flask was added a known volume (100 μl) of 1.0×10^{-3} mol l^{-1} TAAlPc solution, followed by a known volume of 1 mol l^{-1} HCl solution, sodium sulfite and a suitable aliquot of standard formaldehyde solution (or sample to be determined), and the mixture was diluted to 10.0 ml with water. The relative fluorescence intensities of the reagent blank (F_0) and the sample solution (F) were measured at 686 nm with excitation at 610 nm. A calibration graph of the fluorescence quenching (F_0/F) *vs*. the concentration of formaldehyde was plotted.

3. Results and discussion

3.1 Structure and spectral characteristics of TAAlPc

The structure of TAAlPc is given in Fig. 1. The peripheral benzene rings of phthalocyanine are substituted by four amino groups. The addition of peripheral amino groups greatly increases the solubility of the phthalocyanine moiety because the symmetry of the phthalocyanine ring is reduced and thus the aggregation decreases. Hence, a stock solution of TAAlPc could be prepared at a concentration of 1.0×10^{-3} mol l^{-1}. With the addition of hydrochloric acid to the solution of TAAlPc, the absorption spectrum of TAAlPc changed markedly (see Fig. 2). The absorption peak in the long-wavelength region (Q-band) was blue-shifted by about 60 nm and considerable hyperchromism occurred. This can be explained by protonation of the amino groups on the phthalocyanine.[35] It was found that when a small amount of Na_2SO_3 was added, the absorption of TAAlPc in the Q-band region decreased slightly, but a marked decrease was observed when a large amount of Na_2SO_3 was added. A similar hypochromic effect of HCHO on TAAlPc was also observed, and the absorption spectrum of TAAlPc in the Q-band region was obviously red-shifted when large amounts of HCHO were present. When small amounts of Na_2SO_3 and HCHO were present together, the Q-band absorption showed hypochromism and red-shifted markedly, the peak wavelength of the absorption band being even longer than that of TAAlPc in neutral media. The phenomena described above revealed that a reaction occurred between SO_3^{2-}, HCHO and TAAlPc, resulting in the variation of the molecular absorption spectrum of

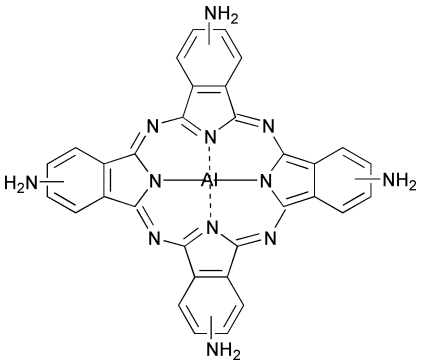

Fig. 1 Structure of TAAlPc.

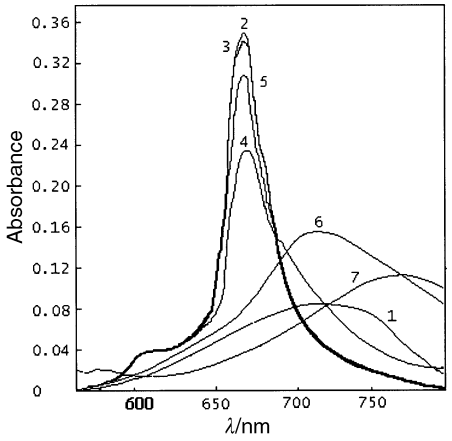

Fig. 2 Absorption spectra:1. TAAlPc; 2. TAAlPc +1.2 mol l^{-1} HCl; 3. TAAlPc +1.2 mol l^{-1} HCl + 8.0×10^{-3} mol l^{-1} Na_2SO_3; 4. TAAlPc +1.2 mol l^{-1} HCl + 0.24 mol l^{-1} Na_2SO_3; 5. TAAlPc +1.2 mol l^{-1} HCl + 2.0×10^{-4} g mL^{-1} HCHO; 6. TAAlPc +1.2 mol l^{-1} HCl + 0.2 g/mL HCHO; 7. TAAlPc + 1.2 mol l^{-1} HCl + 2.0×10^{-4} g/mL HCHO + 8.0×10^{-3} mol l^{-1} Na_2SO_3. [TAAlPc] = 1.0×10^{-5} mol l^{-1}; [HCl] = 1.2 mol l^{-1}.

TAAlPc, and this reaction was dramatically promoted when both SO_3^{2-} and HCHO were present.

As indicated in an earlier paper,[35] the absorption and fluorescence spectra of TAAlPc are greatly affected by the acidity of the environment. Thus, the hypochromism of TAAlPc in the presence of large amounts of SO_3^{2-} is attributed to the decrease of acidity in the system caused by SO_3^{2-}.

To discuss the mechanism of the reaction between SO_3^{2-}, HCHO and TAAlPc, we can find clues from the reaction between pararosaniline, HCHO and SO_3^{2-}, which has become the principle of a classical method for the determination of formaldehyde as well as sulfur dioxide/sulfite.[6,36] Historically, many models were established to explain the reaction between pararosaniline, HCHO and SO_3^{2-}. Three of them, established by Nauman *et al.*,[37] Miksch[6] and Dasgupta *et al.*,[36] seem to be representative, with the last being generally accepted nowadays. This model suggests that pararosaniline, bearing free amino groups in each of its three phenyl groups, reacts with formaldehyde to form a carbinolamine adduct, followed by direct nucleophilic attack by hydrogensulfite to form the final chromophore which is an alkylsulfonic acid. We believe that the reaction between TAAlPc, formaldehyde and sulfite occurs by a similar process since there are also free amino groups connected to phenyl groups in the structure of TAAlPc and the reaction was also carried out in a strongly acidic medium.

The excitation and emission spectra of TAAlPc in the studied system are given in Fig. 3 and 4, respectively. The spectra showed that the fluorescence quenching of the system was proportional to the concentration of formaldehyde. In previous work,[35] we found that TAAlPc was almost non-fluorescent in neutral medium but was fluorescent in acidic medium. This phenomenon could be explained by the principle that electron-withdrawing groups are beneficial to the fluorescence of TAAlPc. Based on the previous work, we suggest that the formation of the alkylsulfonic acid derivative of TAAlPc lowers the positive charge on the amino groups, leading to a decrease of the fluorescence. In the recommended procedure, the final concentration of sulfite was about 200 times lower than that of HCl; hence, the acidity change of the solution caused by sulfite is negligible and can be ignored.

It should be noted that in order to avoid the interference from second-order scattering, the excitation spectrum was obtained by measuring the emission at 670 nm.

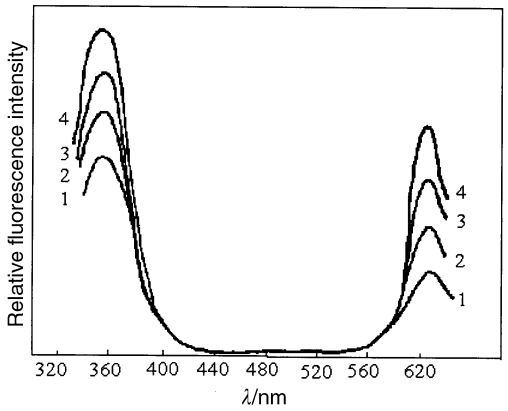

Fig. 3 Excitation spectra of TAAlPc: TAAlPc, 1.0×10^{-5} mol l^{-1}; Na_2SO_3, 8.0×10^{-4} mol l^{-1}; pH 1.2. 4 → 1. HCHO: 0, 15.8, 47.4, 79.0 ng ml^{-1}.

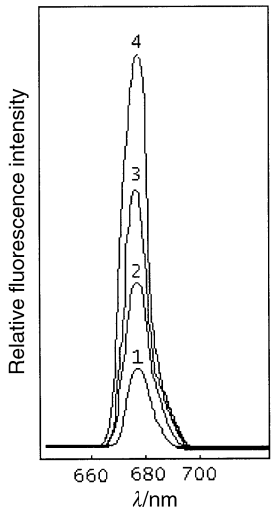

Fig. 4 Emission spectra of TAAlPc: TAAlPc, 1.0×10^{-5} mol l^{-1}; Na_2SO_3, 8.0×10^{-4} mol l^{-1}; pH 1.2. 1 → 4. HCHO: 0, 15.8, 47.4, 79.0 ng ml^{-1}.

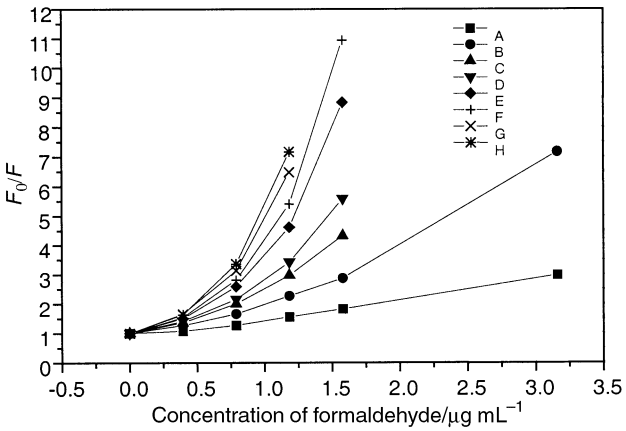

Fig. 5 Quenching effect of different reaction times. A → H: 0, 15, 30, 40, 50, 60, 70, 80 min.

Table 1 Tolerance of foreign substances

Foreign substance	Tolerance limit ($W_{species}/W_{formaldehyde}$)	Relative error (%)
Methane	5.5×10^3	−4.0
Ethanol	3.3×10^5	−3.0
Formic acid	2760	−4.0
Acetic acid	5520	−2.0
Urea	1380	−1.6
Glucose	7000	−2.5
Acetone	7000	−2.5
Acetaldehyde	3.2	+5.0
Glyoxal	38	+5.0
Benzaldehyde	7	+2.0
$Pb(NO_3)_2$	1000	+2.0
Cd^{2+}	140	+4.0
Mn^{2+}	764	+0.2
Mg^{2+}	2.4×10^3	+4.0
Co^{2+}	2050	+0.2
Al^{3+}	938	+2.8
Ca^{2+}	1111	−2.6
Ni^{2+}	82	−2.0
Cu^{2+}	88	+1.0
Zn^{2+}	400	+1.9
Br^-	1110	+5.0
Phenol	15 000	−4.5
I^-	1800	+0.8
Hg^{2+}	60	+5.0
Fe^{3+}	78	+5.0
NO_2^-	0.5	+2.0

Table 2 Analytical parameters for the determination of formaldehyde

Linear range/μg ml^{-1}	Linear regression equation	LOD/ng ml^{-1}	r
0.040–1.19	$y = 0.5412 + 4.008x$	7.5	0.9964

Table 3 Determination of formaldehyde in real samples

Sample	Formaldehyde found: Proposed method (n = 3)	Formaldehyde found: Chromotropic acid method (n = 3)	Added/μg (n = 3)	Found[c]/μg	Recovery (%)
Phenol–formaldehyde resin	(0.74 ± 0.09)[a]	(0.69 ± 0.04)[a]	1.60	1.53	96
Air of animal specimens room	(11.20 ± 0.12)[b]	(10.80 ± 0.08)[b]	1.60	1.56	98

[a] Values in %. [b] Values in μg l^{-1}. [c] Mean of three determinations.

3.2 Optimization of the general procedure

The experimental results indicated that the maximum fluorescence quenching was produced when the concentration of TAAlPc was in the range from 2.5×10^{-6} to 2.0×10^{-5} mol l^{-1}. In this work, a TAAlPc concentration of 1.0×10^{-5} mol l^{-1} was chosen. The effect of pH on the fluorescence quenching reached a maximum in the range 0.9–1.5; therefore, a pH of 1.2 was selected and obtained by adding 0.75 ml of 1 mol l^{-1} HCl solution to 10 ml of the final solution. The effect of the concentration of sulfite on the fluorescence quenching of the system was also investigated and the results showed that the fluorescence quenching reached a maximum in the concentration range from 4.0×10^{-4} to 4.0×10^{-3} mol l^{-1}, hence, a sulfite concentration of 8.0×10^{-4} mol l^{-1} was chosen.

The influence of reaction time on the fluorescence quenching of the system was studied and the calibration graphs for different reaction times are shown in Fig. 5. It can be seen that the complex formation proceeds relatively slowly. The effect of temperature was investigated at 4, 28 and 45 °C, respectively; the results showed that no obvious effect was observed on the fluorescence quenching at low concentrations of formaldehyde, but the quenching clearly increased with the increase of temperature at high concentrations of formaldehyde. The results described above indicated that the sensitivity of the method increased with the increase of reaction time and temperature, but the linear range became narrower. As a compromise, a 30 min reaction time and room temperature (~28 °C) were chosen.

From the change of absorption spectrum before and after the reaction and the experimental results obtained from the study of the effect of temperature on the fluorescence quenching at low concentrations of formaldehyde, it can be inferred that the fluorescence quenching is a static process. However, the results in Fig. 5 and the study of effect of the temperature on the fluorescence quenching at high concentrations of formaldehyde suggest a dynamic quenching mechanism. Based on all the above-mentioned results, it could be that a mixed (static/dynamic) quenching mechanism is in effect.

3.3 Interference of foreign substances

The effects of various foreign substances on the determination of 80.0 ng ml^{-1} formaldehyde by the described procedure were studied. The results are shown in Table 1. It can be seen that the method has a high selectivity; compounds such as methanol, urea, glucose and acetone and common inorganic ions do not interfere with the measurement under the conditions given. However, some carbonyl compounds, such as acetaldehyde, glyoxal and benzaldehyde, and the NO_2^- ion seemed to show low tolerance levels.

3.4 Calibration graph

The calibration graph for the determination of formaldehyde, shown in Fig. 5, was constructed under the optimum conditions and exhibited a linear relationship between the extent of fluorescence quenching and the concentration of formaldehyde. All the analytical parameters are presented in Table 2.

The limit of detection (LOD) was given by the equation LOD = Ks_0/S, where K is a numerical factor chosen according to the confidence level desired, s_0 is the standard deviation of the blank measurements (n = 9) and S is the sensitivity of the calibration graph. Here a value of 3 for K was used.

3.5 Application to the determination of formaldehyde in real samples

The contents of free formaldehyde in a phenol–formaldehyde resin and in the air of an animal specimens room were determined by the proposed method and the results were compared with those obtained by the chromotropic acid method[38] (see Table 3). It can be seen that the values obtained by the two methods are in good agreement.

4. Conclusion

A new fluorimetric method for the determination of formaldehyde is presented. The method is rapid, simple, sensitive and of good selectivity. Further work will be attempted to determine SO_2 and NO_x in the air of some pollution spots.

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References

1 X. Y. Tang, *Atmosphere Environmental Science*, High Education Press, Beijing, 1990.
2 T. Staffelbach, A. Neftel, B. Stauffer and D. Jacob, *Nature (London)*, 1991, **349**, 603.
3 L. Gamizgracia and M. D. L. Decastro, *Analyst*, 1999, **124**, 1119.
4 P. Richter, M. D. L. Decastro and M. Valcarcel, *Anal. Lett.*, 1992, **25**, 2279.
5 K. N. Ramachandran and V. K. Gupta, *Chem. Anal. (Warsaw)*, 1993, **38**, 513.
6 R. R. Mlksch, D. W. Anthon, L. Z. Fanning, C. D. Hollowell, K. Revzan and J. Glanville, *Anal. Chem.*, 1981, **53**, 2118.
7 A. A. Ensafi and S. Abassi, *Fresenius' J. Anal. Chem.*, 1999, **363**, 376.
8 A. Afkhami and M. Rezaei, *Microchem. J.*, 1999, **63**, 243.
9 M. Possanzini and V. Dipalo, *Chromatographia*, 1997, **46**, 235.
10 A. Medvedovici, V. Daid, F. David and P. Sandra, *Anal. Lett.*, 1999, **32**, 581.
11 E. Cotsaris and B. C. Nicholson, *Analyst*, 1993, **118**, 265.
12 J. Kaminski, A. Satwal and S. Mahadevan, *J. Liq.Chromatogr.*, 1993, **16**, 521.
13 J. B. Deandrade and R. L. Tanner, *Atmos. Environ., Part A*, 1992, **26**, 819.
14 S. V. Bolta, L. Zupancickralj and J. Marse, *Chromatographia*, 1998, **48**, 95.

15 W. Tashkov, *Chromatographia*, 1996, **43**, 625.
16 M. Dalene, P. Persson and G. Skarping, *J. Chromatogr.*, 1992, **626**, 284.
17 G. Zurek and U. Karst, *J. Chromatogr.*, 1999, **864**, 191.
18 Y. Maeda, X. C. Hu, S. Iton, M. Kitano, N. Takenaka, H. Bandow and M. Munemori, *Analyst*, 1994, **119**, 2237.
19 K. Takeuchi, H. Takahashi, S. Kutsuna and T. Ibusuki, *Bunseki Kagaku*, 1993, **42**, 439.
20 X. U. Lu, M. G. Lu and F. Yin, *Anal. Lett.*, 1992, **25**, 2073.
21 W. H. Chan and Y. Y. Xie, *Anal. Chim. Acta*, 1997, **349**, 349.
22 W. H. Chan and Y. Y. Xie, *Anal. Chim. Acta*, 1997, **339**, 173.
23 W. H. Chan, W. C. Chung and P. X. Cai, *Analyst*, 1995, **120**, 2233.
24 N. Kiba, L. Sun, S. Yokose, M. T. Kazue and T. T. Suzuki, *Anal. Chim. Acta*, 1999, **378**(1–3), 169.
25 Q. J. Fan and P. K. Dasgupta, *Anal. Chem.*, 1994, **66**, 551.
26 T. Nash, *Biochem. J.*, 1953, **55**, 416.
27 S. Belman, *Anal. Chim. Acta.*, 1963, **29**, 120.
28 D. Shen and P. K. Dasgupta, *Environ. Sci. Technol.*, 1987, **21**, 581.
29 S. B. Johnson, C. M. Terry, T. E. Lister and D. Johnson, *Anal. Chem.*, 1999, **71**, 4030.
30 W. R. Summers, *Anal. Chem.*, 1990, **62**, 1397.
31 S. Houdier, S. Perrire, E. Defraneq and M. Legrand, *Anal. Chim. Acta*, 2000, **412**(1–2), 21.
32 S. Houdier, M. Legrand, D. Boturyn, S. Croze, E. Defrancq and J. Lhomme, *Anal. Chim. Acta*, 1999, **382**, 253.
33 S. T. Girousi, E. E. Golia, A. N. Voulgarapoulos and A. J. Maroulis, *Fresenius' J. Anal. Chem.*, 1997, **358**, 667.
34 J. B. Liu, F. Q. Zhao and Y. Zhao, *Acta Phys.-Chim. Sin.*, 1996, **12**, 491.
35 D. H. Li, Q. Y. Chen, Q. Z. Zhu, F. Li, H. Zheng, S. Y. Yang and J. G. Xu, *Talanta*, 1999, **49**, 745.
36 P. K. Dasgupta, K. Decesare and J. C. Ullrey, *Anal. Chem.*, 1980, **52**, 1912.
37 R. V. Nauman, P. W. West, F. Iron and G. C. Gaeke, *Anal. Chem.*, 1960, **32**, 1307.
38 G. Schlesinger and S. L. Miller, *J. Am. Chem. Soc.*, 1973, **95**, 3729.