เอกสารอ้างอิง

1. วิรัช เรืองศรีตระกูล. 2548, **โฟลอินเจคชันที่ใช้การตรวจวัดแบบเคมิลูมิเนสเซนซ์สำหรับการวิเคราะห์เภสัชภัณฑ์**. ขอนแก่น: ภาควิชาเคมี คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น.
2. J. Ruzicka and E. H. Hansen,1988, Flow Injection Analysis, 2nd ed., Wiley, New York.
3. E. H. Hansen, 30 years of flow injection analysis-And passing the torch, *Anal. Chim. Acta*, 600 (2007) 4.
4. เกตุ กรุดพันธ์, 2539, **เทคนิคการวิเคราะห์** Flow Injection Analysis. วารสารการไฟฟ้าแห่งประเทศ ไทย. 5 : 29-40,
5. Flowinstrument. no date . Flow injection analysis. [online]. Available http://www. flowinstrument.com. (November 20, 2007).
6. Informavorld. no date. On-line separation. (online).Available http://www.informaworld.com (November 20, 2007)
7. Globalfia, no date . Gas diffusion. (online).Available http://www.globalfia.com (November 20, 2007).
8. H. Baadenhuijsen and H.E.H. Seuren- Jacobs, Determination of total CO_2 in plasma by automated flow-injection analysis, *Clin. Chem.*, 25 (1979) 443.
9. สุเมธ บุญเกิด. ม.ป.ป. **การแยกสารโดยวิธี** Pervaporation. [ระบบออนไลน์]. แหล่งที่มา http://www.gpo.or.th . (20 พฤศจิกายน 2550)
10. เกตุ กรุดพันธ์, 2539, **เอกสารประกอบการประชุมปฏิบัติการเคมิเชิงวิเคราะห์ด้วยเครื่องมือ**. เชียงใหม่: ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่.
11. E. N. Harvey,1957, A History of Luminescence from the Earliest Time until 1900, The American Philosophical Society, Philadelphia.
12. L. L. Zamora, Y. F. Mestre, M. J. D. Duart, G. M. A. Fos, R. G. Domenech, J. G. Alvarez and J. M. Calatayud, Prediction of the chemiluminescent behavior of pharmaceuticals and pesticides, *Anal. Chem.*, 73 (2001) 4301.

13. A. A. Alwarthan and A. Townshend, Chemiluminescence determination of bufrenorphine hydrochloride by flow injection analysis, *Anal. Chim. Acta*, 185 (1986) 635.

14. A. M. Garcia-Campana, W.R.G. Baeyens, 2001, Chemiluminescence in Analytical Chemistry , *Marcel Dekker*.

15. เวณิกา เบ็ญจพงษ์.ม.ป.ป. **สารฟอกขาวในอาหาร**. [ระบบออนไลน์]. แหล่งที่มา http://wave.prohosting.com (20 พฤศจิกายน 2550)

16. FDA. no date . Prescription drugs containing sulfite. [online]. Available http://accessdata.fda.gov. (April 1,2008)

17. กรมควบคุมมลพิษ. ม.ป.ป. **ศูนย์ข้อมูลวัตถุอันตรายและเคมีภัณฑ์** (MSDS). [ระบบออนไลน์]. แหล่งที่มา http://msds.pcd.go.th. (20 พฤศจิกายน 2550)

18. ศูนย์ข้อมูลวัตถุอันตรายและเคมีภัณฑ์.ม.ป.ป. Disodium sulfite. [ระบบออนไลน์]. แหล่งที่มา http://chemtrack.trf.or.th. (20 พฤศจิกายน 2550)

19. ศูนย์ข้อมูลวิทยา.ม.ป.ป. **สารฟอกสีอันตราย**. [ระบบออนไลน์]. แหล่งที่มา http://www.webdb.msds.pcd.go.th. (20 พฤศจิกายน 2550)

20. หมอชาวบ้าน. ม.ป.ป. **สารฟอกขาวในอาหาร**. [ระบบออนไลน์]. แหล่งที่มา http://www.doctor.or.th. (20 พฤศจิกายน 2550)

21. Association of Official Analytical Chemists. 1990. Official Methods of Analysis: Food Composition, Additives, Natural Contaminants, 15th ed., AOAC, Virginia.

22. S. S. M. Hassan , M. S. A. Hamza and A. H. K. Mohamed, A novel spectrophotometric method for batch and flow injection determination of sulfite in beverages, *Anal. Chim. Acta*, 570 (2006) 232.

23. M. Zhao, D. B. Hibbert and J. J. Gooding, Determination of sulfite in beer samples using an amperometric fill and flow channel biosensor employing sulfite oxidase, *Anal. Chim. Acta*, 556 (2006) 195.

24. I. G. Casella and R. Marchese, Sulfite oxidation at a platinum glassy carbon electrode determination of sulfite by ion exclusion chromatography with amperometric detection, *Anal. Chim. Acta*, 311 (1995) 199.

25. J. S. Redinha, C. Paliteiro and J. L. C. Pereira, Determination of sulfide by square-wave polarography, *Anal. Chim. Acta*, 351 (1997) 115.

26. C. Giuriati , S. Cavalli , A. Gorni , D. Badocco and P. Pastore, Ion chromatographic determination of sulfide and cyanide in real matrices by using pulsed amperometric detection on a silver electrode, *J. Chromatogr. A*, 1023 (2004) 105.

27. G. Jankovskiene, Z. Daunoravicius and A. Padarauskas, Capillary electrophoretic determination of sulfite using the zone-passing technique of in-capillary derivatization, *J. Chromatogr. A*, 934 (2001) 67.

28. A. Isaac, C. Livingstone, A. J. Wain, R.G. Compton and J. Davis, Electroanalytical methods for the determination of sulfite in food and beverages, *Anal. Chem.*, 25 (2006) 589.

29. A. Safavi, O. Moradlou and S. Maesum, Flow injection analysis of sulphite by gas-phase molecular absorption UV/VIS spectrophotometry, *Talanta*, 62 (2004) 51.

30. O. Fatibello - Filho and I. da Cruz Vieira, Flow injection spectrometric determination of sulfite using a crude extract of sweet potato root (*Iponoea batatas* (L.) Lam.) as a source of polyphenol oxidase, *Anal. Chim. Acta*, 354 (1997) 51.

31. S. S. M. Hassan , S. A. Marei , I. H. Badr and H. A. Arida, Flow injection analysis of sulfite ion with apotentiometric titanium phosphate–epoxy based membrane sensor, *Talanta*, 54 (2001) 773.

32. H. Meng, F. Wu, Z. He and Y. Zeng, Chemiluminescence determination of sulfite in sugar and sulfur dioxide in air using Tris(2,2%-bipyridyl)ruthenium(II)-permanganate system, *Talanta*, 48 (1999) 571.

33. F. Wu, Z. He, H. Meng and Yun'e Zeng, Determination of sulfite in sugar and sulfur dioxide in air by chemiluminescence using the $Ru(bipy)_3^{2+}$ –$KBrO_3$ system, *Analyst*, 123 (1998) 2109.

34. Z. He, F. Wu, H. Meng, L. Yuan, G. Song and Y. Zeng, Chemiluminescence determination of sulfite in sugar and sulfur dioxide in air using $Ru(bipy)_3^{2+}$ - $K_2S_2O_8$ system, *Anal. Sci.*, 14 (1998) 737.

35. W. Qin, Z. Zhangb, and C. Zhang, Chemiluminescence flow system for the determination of sulfite, *Fresenius J. Anal. Chem.*, 361 (1998) 824.

36. Z. He, F. Wu, H. Meng, L. Yuan, Q. Luo and Y. Zeng, Chemiluminescence determination of sulfur dioxide in air using tris(1,10-Phenanthroline) ruthenium-KIO_4 system, *Anal. Lett.*, 32 (1999) 401.

37. Y. Huang, C. Zhang, X. Zhang and Z. Zhang, Chemiluminescence of sulfite based on auto-oxidation sensitized by rhodamine 6G, *Anal. Chim. Acta,* 391 (1999) 95.

38. N.T.K. Thanh, L.G. Decnop-Weever and W.T. Kok, Determination of sulphite in wine by flow injection analysis with indirect electrochemical detection, *Fresenius J. Anal. Chem.*, 349 (1994) 469.

39. J. Carinhanha, C. Santos and M. Korn, Exploiting sulphide generation and gas diffusion separation in a flow System for indirect sulphite determination in wines and fruit juices, *Microchim. Acta*, 153 (2006) 87.

40. L.G. Decnop-Weever and J.C. Kraak, Determination of sulphite in wines by gas diffusion flow injection analysis utilizing spectrophotometric pH-detection, *Anal. Chim. Acta*, 337 (1997) 125.

41. C.S. Tavares Arau jo, J. Lira de Carvalho, D. Ribeiro Mota, C.L. de Arau jo and N.M.M. Coelho, Determination of sulphite and acetic acid in foods by gas permeation flow injection analysis, *Food Chem.*, 92 (2005) 765.

42. R. L. Bonifácio and N. Coichev, Chemiluminescent determination of sulfite traces based on the induced oxidation of Ni(II)/tetraglycine complex by oxygen in the presence of luminol: mechanistic considerations, *Anal. Chim. Acta*, 517 (2004) 125.

43. J. M. Lin and T. Hobo, Flow-injection analysis with chemiluminescent detection of sulphite using Na_2CO_3-$NaHCO_3$-Cu^{2+} system, *Anal. Chim. Acta*, 323 (1996) 69.

44. X. Su, W. Wei, L. Nie and S. Yao, Flow injection determination of sulfite in wines and fruit juices by using a bulk acoustic wave impedance sensor coupled to a membrane separation technique, *Analyst*, 123 (1998) 221.

45. J. L. Adcock, P. S. Francis and N. W. Barnett, Acidic potassium permanganate as a chemiluminescence reagent-A review, *Anal. Chim. Acta*, 601 (2007) 36.

46. H. Sulistyarti, T. J. Cardwell, M.D. L. Castro and S. D. Kolev, On-line determination of cyanide in the presence of sulfide by flow injection with pervaporation, *Anal. Chim. Acta*, 390 (1999) 133.

47. N. W. Barnett, B. J. Hindson, P. Jones and T.A. Smith, Chemically induced phosphorescence from manganese(II) during the oxidation of various compounds by manganese (III), (IV) break and (VII) in acidic aqueous solutions, *Anal. Chim. Acta*, 451 (2002) 181.

48 S. Satienperakul , T. J. Cardwell , S. D. Kolev , C. E. Lenehan and N. W. Barnett, A sensitive procedure for the rapid determination of arsenic(III) by flow injection analysis and chemiluminescence detection, *Anal. Chim. Acta*, 554 (2005) 25.

49. N. Anastos, N. W. Barnett, B. J. Hindson1, C. E. Lenehan and S. W. Lewis, Comparison of soluble manganese (IV) and acidic potassium permanganate chemiluminescence detection using flow injection and sequential injection analysis for the determination of ascorbic acid in vitamin C tablets, *Talanta*, 64 (2004) 130.

50. N. W. Barnett, B. J. Hindson and S. W. Lewis, Determination of 5-hydroxytryptamine (serotonin) and related indoles by flow injection analysis with acidic potassium permanganate chemiluminescence detection, *Anal. Chim. Acta*, 362 (1998) 131.

51. N. W. Barnett, B. J. Hindson, S. W. Lewis, P. Jones and P. J. Worsfold, Soluble manganese(IV); a new chemiluminescence reagent, *Analyst*, 126 (2001) 1636.

52. D. Nacapricha , P. Sangkarn , C. Karuwan , T. Mantim, W. Waiyawat, P. Wilairat ,T. Cardwell, I.D. McKelvie and N. Ratanawimarnwong, Pervaporation-flow injection with chemiluminescence detection for determination of iodide in multivitamin tablets, *Talanta*, 72 (2007) 626.

53. Y. Ma, M. Zhou, X. Jin, B. Zhang ,H. Chen and N. Guo, Flow–injection chemiluminescence determination of ascorbic acid by use of the cerium (IV)–Rhodamine B system, *Anal. Chim. Acta*, 464 (2002) 289.

54. H. Chen, M. Zhou, X. Jin, Y. Song, Z. Zhang and Y. Ma, Chemiluminescence determination of ultramicro DNA with a flow-injection method, *Anal. Chim. Acta*, 478 (2003) 31.

55. K. Mervartova , M. Polasek and J. M. Calatayud, Sequential injection analysis (SIA) chemiluminescence determination of indomethacin using tris (2,2'bipyridyl) ruthenium(III) as reagent and its application to semisolid pharmaceutical dosage forms, *Anal. Chim. Acta*, 600 (2007) 114.

56. T. Rupasinghe , T. J. Cardwell, R. W. Cattrall , I.D. Potter and S. D.Kolev, Determination of arsenic by pervaporation-flow injection hydride generation and permanganate spectrophotometric detection, *Anal. Chim. Acta*, 510 (2004) 225.

57. I. Papaefstathiou, M.T. Tena and M.D.L. Castro, On-line pervaporation separation process for the potentiometric determination of fluoride in "dirty" samples, *Anal. Chim. Acta*, 308 (1995) 246.

58. S. Y. Sheilheldin, T. J. Cardwell, R. W. Cattrall, M. D. Luque de Castro and S. D. Kolev, Determination of phenol in water by pervaporation-flow injection analysis, *Anal. Chim. Acta*, 419 (2000) 9.

59. S. Satienperakul, S. Y. Sheikheldin , T. J. Cardwell, R.W. Cattrall, M. D. Luque de Castro , I. D. McKelvie and S. D.Kolev, Pervaporation-flow injection analysis of phenol after on-line derivatisation to phenyl acetate, *Anal. Chim. Acta*, 485 (2003) 37.

60. แม้น อมรสิทธิ์ และอมร เพชรสม. 2534. **หลักการและเทคนิคการวิเคราะห์เชิงเครื่องมือ**. กรุงเทพฯ: ชวนพิมพ์.

ภาคผนวก

ผลงานตีพิมพ์ที่เป็นผลเกี่ยวข้องที่เกิดจากโครงการวิจัย

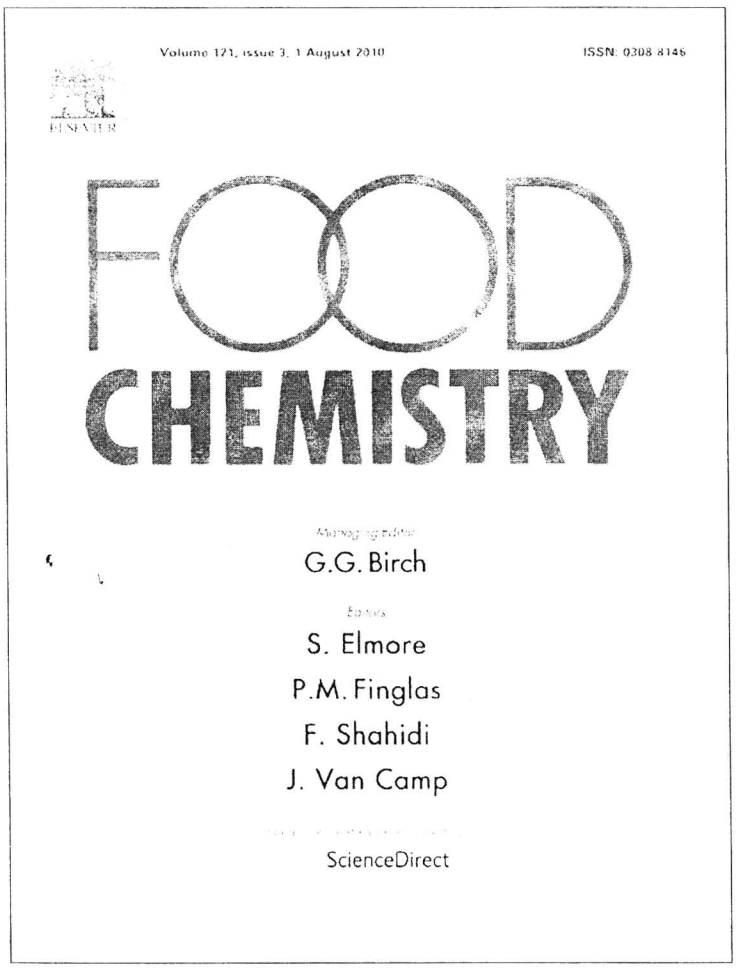
Research article

1. Sakchai Satienperakul, Pornthana Phongdong and Saisunee Liawruangrath, Pervaporation flow injection analysis for the determination of sulphite in food samples utilising potassium permanganate-rhodamine B chemiluminescence detection, *Food Chemistry*, 2010, **121**(3) : 893-898. Impact Factor 2.696

2. Lori Shayne T. Alamo,Tanin Tangkuaram and Sakchai Satienperakul, Determination of Sulfite by pervaporation flow Injection with amperometric detection using copper hexacyanoferrate-carbon nanotube modified carbon paste electrode, *Talanta*. 2010, **81** : 1793–1799. Impact factor: 3.206

Conference paper

1. Sakchai Satienperakul, Saisunee Liawruangrath, Terence J. Cardwell, Novel chemiluminescence flow-injection analysis for the determination of sulphide in aqueous samples, *Luminescence*, 2006, **21**(5) : 290-291, Impact factor 1.317



Food Chemistry 121 (2010) 893–898

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Pervaporation flow injection analysis for the determination of sulphite in food samples utilising potassium permanganate–rhodamine B chemiluminescence detection

Sakchai Satienperakul [a,*], Pornthana Phongdong [a], Saisunee Liawruangrath [b]

[a] Department of Chemistry, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand
[b] Department of Chemistry and Centre for Innovation in Chemistry, Postgraduate Education and Research Program in Chemistry (PERCH-CIC), Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

ARTICLE INFO

Article history:
Received 14 July 2009
Received in revised form 1 January 2010
Accepted 16 January 2010

Keywords:
Sulphite
Flow injection
Pervaporation
Chemiluminescence
Acidic potassium permanganate
Rhodamine B

ABSTRACT

A simple pervaporation flow injection chemiluminescence (PFI-CL) procedure was utilised as an on-line separation for the analysis of contaminated sulphite in food samples. The method involves the injection of standard and/or sulphite sample solutions into a 0.20 M sulphuric acid donor stream. Sulphite is converted to sulphur dioxide and transported to the donor chamber of a pervaporation module. The sulphur dioxide gas then evaporates into the headspace and diffuses across a semi-permeable PTFE membrane into an acceptor stream containing 0.75% (m/v) sodium hexametaphosphate and 1.0 mg L^{-1} rhodamine B in 0.02 M H_3PO_4, which functions as a carrier solution for the chemiluminescence detection. The sulphur dioxide in the acceptor stream merges at a T-piece with a reagent stream consisting of potassium permanganate (8.0×10^{-5} M) prepared in the acidic sodium hexametaphosphate carrier solution. The elicited chemiluminescence intensity of the resulting reaction mixture was measured at a red sensitive photomultiplier tube operated at a voltage of 1.00 kV. Optimal experimental conditions for an on-line determination of sulphite were investigated. The second-order polynomial calibration curve was developed over the concentration range of 0.5–10.0 mg L^{-1} sulphite with a resulting equation of $I = -0.239C^2 + 4.846C - 1.64$, $r^2 = 0.9997$. The detection limit was found to be 0.2 mg L^{-1} with a sampling frequency of 30 h^{-1}. The effects of common anionic and cationic interferences were also investigated. The proposed PFI procedure was successfully applied to the determination of sulphite in different food samples. The PFI data was validated versus standard differential pulse polarography.

1. Introduction

Sulphiting agents (sulphur dioxide, sulphite, hydrogensulphite and metabisulphite) are commonly used as an additive in several foods and beverages for various purposes; e.g. to inhibit undesirable micro-organisms in fermentation industries, to control enzymatic and non-enzymatic browning reactions, and to assist in preserving vitamin C (Hanssen, Marsden, & Norris, 1989). Sulphite has been reported to cause harmful effects in hypersensitive people, exhibiting a wide variety of symptoms from hives, nausea and diarrhoea to respiratory failure and asthmatic attacks. Hence, products containing more than the established threshold sulphite level should be labelled adequately. The US Food and Drug Administration (FDA) requires sulphite declaration on the label of any food in concentrations of 10 μg mL^{-1} sulphite or more (Warner, Diachenko, & Bailey, 2000). This obligation is also compulsory for pre-packed food sold in the UK and the rest of the European Union (Food Standards Agency, 2005/2006).

The Association of Official Analytical Chemists (AOAC, 1990) standard detection method involves an iodimetry or gravimetry (detects sulphate) or polarographic procedure for sulphite determination in foods and beverages. These procedures require an acid distillation to isolate gaseous sulphur dioxide from sample matrices prior to analysis. Normally, the conventional titrimetric method suffers from poor precision and large time consumption during the acid distillation step, while the polarographic chemicals are highly toxic and harmful.

Although the official methods are available to determine sulphite and sulphur dioxide in food samples, newer methods appear in recent literature. One especially searches for a simple and sensitive method which can be used for a broad range of complex samples (Ruiz-Capillas & Jiménez-Colmenero, 2009) and which can be easily automated for an on-line separation. Gas diffusion flow injection analysis (GD-FI) is one choice for determining sulphite and sulphur dioxide in food and beverages. This method is based on

* Corresponding author. Tel.: +66 53 873544; fax: +66 53 878225.
E-mail address: sakchais@mju.ac.th (S. Satienperakul).

0308-8146/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2010.01.013

changes in pH or conductivity due to the protolytic reaction of diffused sulphur dioxide trapped in an electrolyte acceptor solution. The changes caused by the protolytic reaction can be monitored by spectrophotometry (Hassan, Hamza, & Mohamed, 2006), conductivity (Tavares Araujo, Lira de Carvalho, Ribeiro Mota, Araujo, & Coelho, 2005), potentiometry (Azevedo, Araki, Toma, & Angnes, 1999), amperometry (Chinvongamorn, Pinwattana, Praphairaksit, Imato, & Chailapakul, 2008), or by using sensitive detection such as bulk acoustic wave impedance (Su & Wie, 1998), or even chemiluminescence detection (Lin & Hobo, 1996). Not only does a major problem with these methods arise from carbon dioxide uptake interferences, but membrane failure through contact with dirty samples also leads to serious limitations with the instrumental set-up for sample pre-treatment purposes.

Gas diffusion techniques suffer from two major drawbacks: potential clogging of the membrane pores by suspended particles or high molecular weight compounds in food matrices, and degradation of the membrane through contact with samples containing corrosive or surface active agents. Both problems can be overcome by the separation technique called *pervaporation* which can be defined as the integration of continuous evaporation and gas permeation processes in the same module (Amador-Hernandez & Luque de Castro, 2000). This technique is based on converting and/or evaporating the analyte in the donor stream to a molecular gas. This gas then diffuses across the air gap between the membrane and the liquid level in the donor chamber, and then through the membrane into the acceptor stream where detection takes place. The constant air gap in the donor chamber prevents the direct contact between the sample and the membrane.

Thus, food and beverage products consisting of different aggregation and physical properties in the sample matrices can be analysed by using versatile designs of the pervaporation manifold in quality control steps to save time and labour costs. Various examples of pervaporation flow injection (PFI) in food quality control have been reported including the determination of sulphide in Kraft liquors (Papaefstathiou, Luque de Castro, & Valcarcel, 1996), sulphur dioxide in wines (Mataix & Luque de Castro, 1998), diacetyl (Izquierdo-Ferrero, Romero, & Luque de Castro, 1997), urea (Gonzalez-Rodroguez, Perez-Juan, & Luque de Castro, 2002a, 2002b), ammonia in beers (Wang, Cardwell, Cattrall, Luque de Castro, & Kolev, 2003), and methanol and iron combined in vinegar (Gonzalez-Rodroguez et al., 2002a, 2002b). These are testaments to its versatility and robustness.

There is a drawback to using pervaporation in comparison to gas diffusion, where the slower mass transfer process results in higher detection limits. This drawback can be compensated to a considerable extent by using membranes of high permeability, applying a stopped flow mode (Satienperakul et al., 2003), using ultrasound-assisted extraction (George, Pereira, Al Massum, Kolev, & Ashokkumar, 2008) or using a sensitive detection method such as chemiluminescence (Nacapricha et al., 2007).

According to the recent literature reviews (Adcock, Francis, & Barnett, 2007; Hindson & Barnett, 2001), using acidic potassium permanganate is a simple and promising chemiluminescence reagent for the determination of inorganic sulphur compounds with various fluorescence enhancers. Oxidation of sulphite with acidic potassium permanganate demonstrates that the chemiluminescence intensity can be enhanced in the presence of either riboflavin phosphate or brilliant sulfaflavine. (Yamada, Nakada, & Suzuki, 1983). Adcock, Francis, and Barnett (2009) also found that the oxidation of sulphite with either acidic cerium (IV) or permanganate can be enhanced by 3-cyclohexylamino-propanesulphonic acid (CAPS).

In the present paper, the inherent selectivity of the membrane separation process of PFI with sensitive $KMnO_4$ chemiluminescence detection is utilised with rhodamine B added as a common fluorescent enhancer for simple on-line separation and determination of contaminated sulphite in food samples.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical reagent (AR) grade and were used as received. Water was purified using a compact Ultra pure deionised water system (18.2 MΩ cm, Millipore, France) and used for all solution preparations. Potassium permanganate stock solution (1.0×10^{-2} M) was prepared by dissolving 0.1587 g of $KMnO_4$ (Ajax, Australia) in 100 mL deionised water and stored in a refrigerator for one week. A 0.20 M sulphuric acid solution, used as the donor stream, was prepared by appropriate dilution of concentrated sulphuric acid (Merck, Germany). The acceptor stream solution containing 0.75% (m/v) sodium hexametaphosphate was prepared daily by dissolving the appropriate amount of sodium hexametaphosphate in 0.02 M phosphoric acid (Merck, Germany) solution and the appropriate amount of rhodamine B (Fluka, Switzerland) was added to make the final concentration of 1.0 mg L^{-1}.

The reagent stream solution, acidic potassium permanganate (8.0×10^{-5} M), was prepared fresh daily by making the appropriate dilution of the $KMnO_4$ stock solution in 0.75% (m/v) sodium hexametaphosphate solution in 0.02 M phosphoric acid. All working solutions were degassed by bubbling nitrogen through the solution before use.

Sulphite stock solution (1000 mg L^{-1}) was prepared by dissolving 0.1589 g of anhydrous Na_2SO_3 in 100 mL of deionised water and standardised by titrating with iodine (AOAC, 1990). This stock solution was stored in a sealed container at 4 °C when not in use. Standard solutions of sulphite (0.8–4.0 mg L^{-1}) were prepared as needed by making appropriate dilutions of the stock sulphite solution (1000 mg L^{-1}) with the deionised water.

2.2. Apparatus

The PFI-CL system set-up, shown schematically in Fig. 1, contains two peristaltic pumps with rate selectors (Minipuls 3, Gilson, France), a sample injection valve (V-450, Upchurch Scientific, WA, USA), and a custom built Perspex pervaporation unit with hexagonal shaped channels similar to the one described elsewhere (Sulistyarti, Cardwell, Luque de Castro, & Kolev, 1999). A single layer of glass beads (3 mm diameter, Selby-Biolab, Australia) was used to cover the floor of the donor chamber in the pervaporation unit to help maintain a constant liquid level for achieving good reproducibility. A circular semi-permeable PTFE membrane (40 mm diameter, 40 mm diameter, 1.5 mm thickness, Trace Biotech, Germany) was sandwiched between the donor and acceptor chambers of the pervaporation unit. The remaining flow-through sections of the flow system (i.e. sample loop and connection tubes) consist of PTFE tubing (0.5 mm i.d).

The CL signal was monitored in a custom built flow-through luminometer, which consisted of a flat spiral coil flow cell (glass tubing i.d. 1.5 mm, spiral coil diameter 25 mm) mounted flush against a red sensitive photomultiplier tube (PMT, Thorn-EMI 9878SB, Electron Tubes, UK), where the chemiluminescence intensity was detected. The operational potential for the PMT was provided by a stable power supply (Thorn-EMI model PM20, Electron Tubes, UK) at a voltage of 1.00 kV. The output of the PMT, proportional to the chemiluminescence intensity, was monitored continuously and displayed by a PC (Pentium IV) via a digital multimeter USB/RS-232 (UT60D, Hong Kong) interfaced with the voltage divider (C637BFN2, Electron Tubes, UK). The UNI-T® UT60D computer

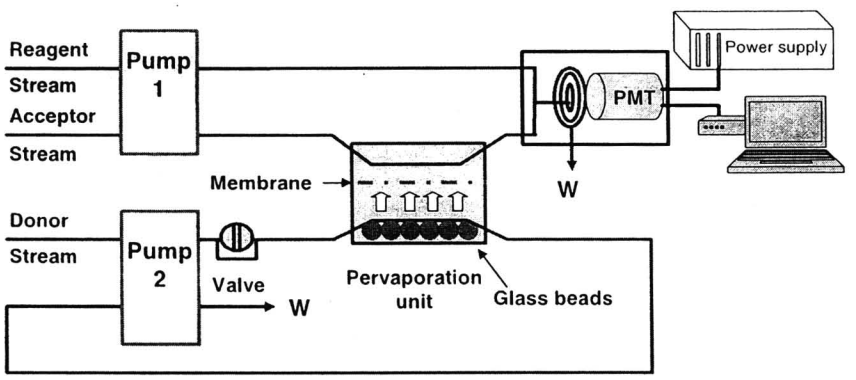


Fig. 1. Schematic diagram of the PFI-CL system used for sulphite determination. Reagent stream (8.0×10^{-5} M $KMnO_4$ in 0.75% sodium hexametaphosphate in 0.02 M H_3PO_4); acceptor stream (1.0 mg L^{-1} rhodamine B in 0.75% sodium hexametaphosphate in 0.02 M H_3PO_4, pH 2.70); donor stream (0.20 M H_2SO_4); injection valve (500 μL sample loop); and PMT, red sensitive photomultiplier tube.

software was used to determine the maximal output potential corresponding to the peak maximum.

The AOAC (1990) standard differential pulse polarographic method (987.04) was performed using a voltammograph (Metrohm Model VA 741, Metrohm, Switzerland). All samples were acid distilled by purging the acidified sample with oxygen-free nitrogen and collecting in an electrolyte-trapping solution (2 M ammonium acetate buffer with 5% ethanol) prior to polarographic analysis.

2.3. Procedures

A 500 μL sample or standard sulphite solution was injected manually via an injection valve into a 0.20 M H_2SO_4 donor stream at the controlled flow rate of 2.0 mL min^{-1}. The resulting mixture of the injected solution with the sulphuric donor stream was transported to the donor chamber of the pervaporation unit where any sulphite present was converted to sulphur dioxide and evaporated into the headspace. The sulphur dioxide gas then diffused across the PTFE membrane into an acceptor solution containing 0.75% (m/v) sodium hexametaphosphate and 1.0 mg L^{-1} rhodamine B in 0.02 M H_3PO_4. The peristaltic pump (Pump 2 in Fig. 1) was employed to both propel and withdraw the donor solution from the pervaporation unit; it also assisted in the maintenance of a constant liquid level in the donor chamber, which plays a crucial role in achieving reproducible results (Luque de Castro & Papaefstathiou, 1998). The liquid was maintained at a level where the glass beads were fully submerged.

The CL detection system was completed by utilising the other two-channel peristaltic pump (Pump 1 in Fig. 1) to propel the acceptor and reagent streams at equal flow rates, with a total flow rate of 2.0 mL min^{-1}. After the sulphur dioxide gas diffuses into the acceptor channel, it is transported to and merges at a T-piece with the reagent stream of potassium permanganate (8.0×10^{-5} M) prepared in the acidic sodium hexametaphosphate solution (0.02 M H_3PO_4). The combined reaction mixture immediately passes through the flat spiral coil flow cell, where the chemiluminescence intensity is detected at the red sensitive photomultiplier tube operated at a voltage of 1.00 kV. The output of the PMT which is proportional to the chemiluminescence intensity was monitored continuously. The analytical signal of the PFI-CL system was the maximal output potential corresponding to the peak maximum.

The AOAC Standard Method (987.04) was used as the reference method. A suitable amount of liquid, homogenised slurry sample, or suitable volume of standard was put into a test tube and acidified with 5 mL of 37% HCl. The collection flasks were prepared by adding 10 mL of acetate buffer. Nitrogen was flushed into the test tube containing the sample through a capillary tube at a flow rate of 250 mL min^{-1} for 15 min. The sample temperature was controlled by placing the test tube in gently boiling water. The collected electrolyte-trapping solution was then analysed by differential pulse polarography.

3. Results and discussion

The method presented in this paper is based on the on-line membrane separation of sulphite via the generation of sulphur dioxide gas in an acidic donor stream. The chemiluminescence emission of sulphite or sulphur dioxide with acidic potassium permanganate is often attributed to the generation of an excited sulphur oxyanion with permanganate (Adcock et al., 2007). It also has been reported that the presence of polyphosphates such as sodium hexametaphosphate provides significant increase in signal intensity from acidic potassium permanganate compared to the use of only acid (Hindson & Barnett, 2001); but this has not previously been used in chemiluminescent reactions involving sulphite (Adcock et al. (2009)). The detection of SO_2 in the acceptor solution was studied during the diffusive sample transport across the membrane, which in turn is influenced by a number of the PFI parameters.

3.1. Optimisation of experimental variables

The flow system parameters that affect the sensitivity and reproducibility of sulphite determination in the PFI system were studied separately. A series of experiments were conducted to establish the optimum analytical variables that give the higher CL sensitivity. The order in which PFI parameters are listed in Table 1 corresponds to the actual order in which the optimisation took place; the range over which each was investigated and its optimal value are depicted. The donor and acceptor streams were optimised independently with the acceptor stream (CL detection) optimised first.

3.1.1. Effect of photomultiplier tube voltage

The effect of the photomultiplier tube (PMT) voltage was investigated in the range 0.80–1.10 kV, noting that the maximum input voltage recommended by the manufacturer was 1.10 kV. For these experiments, 2 mg L^{-1} sulphite standard solutions were injected into a donor stream solution. The potential of the power supply was increased stepwise and the analytical signal was measured after injection of a sulphite solution at each potential step. The noise from the background current was also measured at each potential. It was found that the signal-to-noise ratio reached a

Table 1
Optimised flow system parameters.

Parameters	Range studied	Initial value	Optimal value
PMT voltage (kV)	0.80–1.10	0.95	1
Acceptor stream			
Acid type	$HClO_4$, HNO_3, H_2SO_4, H_3PO_4	H_2SO_4	H_3PO_4
H_3PO_4 concentration (M)	0.01– 0.10	0.05	0.02
$(NaPO_3)_6$ concentration (%, m/v)	0.25–1.25	1	0.75
$KMnO_4$ concentration (M)	1×10^{-5} to 1×10^{-3}	5.0×10^{-4}	8.0×10^{-5}
Total flow rate (mL min^{-1}) (acceptor + reagent stream)	1.0–6.0	2.5	2
Injection volume (μL)	50–1000	300	500
Rhodamine B (mg L^{-1})	0.5–5.0	–	1
Donor stream			
H_2SO_4 concentration (M)	0.05–0.30	0.25	0.2
Flow rate (mL min^{-1})	0.5–3.0	2	2

maximum value at 1.00 kV, which was selected for all subsequent experiments.

3.1.2. Effect of acid composition in the acceptor solution

The influence of the acid media was studied by using 1% (m/v) acidified sodium hexametaphosphate in different acids; $HClO_4$, HNO_3, H_2SO_4 and H_3PO_4 were tested in the range 0.01–0.10 M in order to ascertain which acid was the most suitable. Only sulphuric and orthophosphoric acid gave suitable PFI-CL signals at concentrations in the range of 0.02–0.10 M, but sulphuric acid yielded the lower sensitivity. The concentration-analytical signal profile for orthophosphoric acid was then studied and Fig. 2a shows the maximum CL intensity at a concentration of 0.02 M H_3PO_4. Therefore, 0.02 M orthophosphoric acid was chosen for subsequent studies.

3.1.3. Effect of sodium hexametaphosphate concentration

Evaluation of the sodium hexametaphosphate $(NaPO_3)_6$ concentration over the range 0.25–1.25% (m/v) showed a slight decrease in PFI-CL signal once the sodium hexametaphosphate concentration was greater than 0.75% (m/v). Thus, the concentration of 0.75% (m/v) was selected for subsequent experiments.

3.1.4. Effect of potassium permanganate concentration

Evaluation of the $KMnO_4$ concentration over the range 1.0×10^{-5} to 1×10^{-3} M showed an increase in analytical signal as the $KMnO_4$ concentration increased to 8.0×10^{-5} M. Above this concentration, the PFI-CL signal decreased gradually (as shown in Fig. 2b) mainly due to the intense colour of the permanganate solution. Thus, 8.0×10^{-5} M was selected for all subsequent experiments.

3.1.5. Effect of acceptor and reagent total flow rate

Flow rate is an important parameter in CL detection as the time taken to transfer the excited product into the flow cell is critical for maximum collection of the emitted light (Meste, Zamora, & Calatayud, 2001). The flow rates of the acceptor stream (functioning as the carrier stream) and reagent stream were then optimised in order to obtain satisfactory CL intensity. To simplify the optimisation of the flow rate in the CL detection system, the reagent and acceptor streams flow rates were kept equal in each channel. The effect of the overall flow rate of both streams was studied over the range of 0.5–3.0 mL min^{-1}. The analytical signal increased as the flow rate increased up to 1.0 mL min^{-1}; increasing the flow rate above this point causes the CL intensity to diminish gradually due to the poorer mass transfer occurring in the membrane separation process in the PFI unit. Thus, the maximum CL intensity was obtained from the flow rate of 1.0 mL min^{-1} for each channel; and this total flow rate of 2.0 mL min^{-1} was considered to be optimal using the univariate approach.

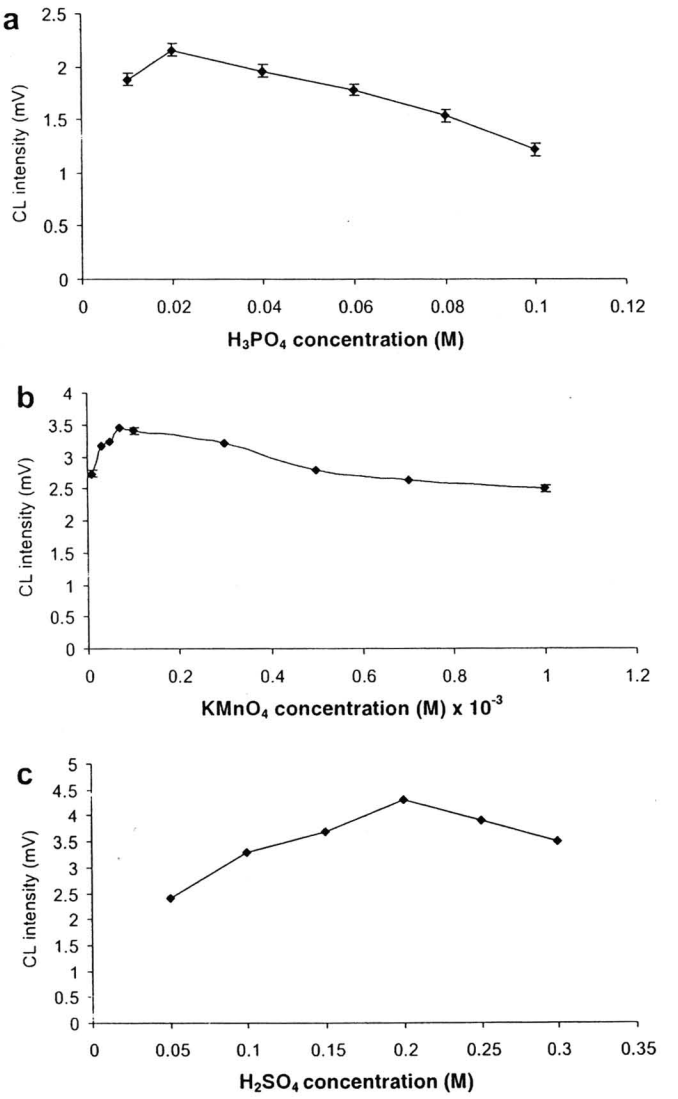


Fig. 2. Variation of CL intensity on (a) orthophosphoric acid concentration; (b) potassium permanganate concentration and (c) sulphuric acid concentration (experimental conditions: refer to Table 1).

3.1.6. Effect of injection volume

It is necessary to optimise the injection volume to achieve the desired sensitivity. The amount of sample injected into the PFI system should be sufficient to permeate though the membrane and permit effective CL reaction. The influence of the sample/standard

volume on the CL intensity was investigated by injecting the standard solution with varying volumes in the range of 50–1000 μL. It was found that the PFI-CL peak height increased with the injection volume up to 500 μL and remained almost constant above this value. At very large sample volumes (>500 μL), peak tailing and unstable baselines were observed due to increases in dispersion. Thus, a volume of 500 μL was selected for all remaining experiments.

3.1.7. Effect of fluorescent sensitisers

Some highly fluorescent compounds have been reported to act as an energy-transfer in the sulphite-permanganate CL reactions with an enhancement of the CL intensity (Adcock et al., 2007; Al-Tamrah, Townshend, & Wheatley, 1987). Selected common fluorophores were examined for obtaining maximum yields in CL intensity. In the present work, 5 mg L^{-1} of quinine, fluorescein and rhodamine B, were included in the acidic polyhexametaphosphate acceptor stream solution to be tested as potential enhancers. It was found that the addition of the first two sensitisers had little or no effect on CL intensity; whereas a significant increase in emission intensity was observed with rhodamine B addition. A previous report by Adcock et al. (2009) claimed that CL intensity from the reaction of permanganate, sulphite and papaverine can be increased (by two orders of magnitude) with the addition of 0.1% (m/v) sodium polyphosphate to the oxidant solution. But according to our investigation, the addition of rhodamine B (1.0 mg L^{-1}) into the polyphosphate acceptor stream results in the increase of CL intensity by three orders of magnitude. Beyond this concentration, the PFI-CL signal decreases gradually with increasing rhodamine B concentration.

3.1.8. Effect of donor stream acid concentration and flow rate

The generation of SO_2 gas takes place in an acidic medium and in most GD-FI sulphite determinations, solutions of sulphuric acid between 0.1 and 0.5 M were used. The evaluation of the H_2SO_4 concentration used as a donor stream over the range 0.05–0.30 M was carried out with the flow rate at 2.0 mL min^{-1}. Fig. 2c shows a variation in the analytical signal with increasing acid concentration, where the maximum signal was obtained at 0.20 M H_2SO_4. The donor stream flow rate was investigated over the range of 0.5–3.0 mL min^{-1} and the maximum CL intensity was obtained at the flow rate of 2.0 mL min^{-1}. This concentration and flow rate were considered to be optimal using the univariate approach.

3.2. Detection limit, sampling rate and linear detection range for the PFI system

Under the optimised working conditions (Table 1), the sample through-put was estimated to be 30 h^{-1}. A series of standard solutions (0.5–10.0 mg L^{-1}) were used to study the linear range of the calibration graph. In common with previous reports (Hindson & Barnett, 2001), the calibration line was not linear over the entire concentration range and the trend is better described by the second-order polynomial equation: $I = -0.239C^2 + 4.846C - 1.64$ ($r^2 = 0.9997$), where I is the CL intensity in mV and C is the concentration expressed in mg L^{-1}.

However, the calibration approximates linearity in the limited range (0.8–4.0 mg L^{-1}) and in this dynamic range, the linear regression equation could be expressed as $I = 3.1C + 0.8976$ ($r^2 = 0.9913$). The limit of detection, defined as three times the standard deviation of the noise was determined to be 0.2 mg L^{-1}.

3.3. Effect of potential interferences

Interferences in the determination of 2.0 mg L^{-1} sulphite using the proposed method were examined. The tolerance limit was taken as the amount which caused an error of ±5% in peak height. The maximum tolerable concentrations for each coexistent substance are shown in Table 2. Most substances which are always found in pickled foods have almost no effect on the determination of high concentration levels of sulphite. However, the serious negative interference was encountered with ethanol at very high concentrations possibly due to the evaporation of ethanol into the acceptor solution diminished CL emission when rhodamine sensitiser being used, similar to previous work reported by Huang, Zhang, Zhang, and Zhang (1999).

3.4. Determination of total sulphite in food samples

The proposed PFI-CL method has been successfully applied to determine the sulphite content in different types of foods and the results were compared with those obtained by the standard AOAC method. In order to perform the PFI-CL analysis under the optimal concentration range, the homogenised slurry samples were diluted with water by a factor of 3–1000. The sulphite content was calculated from the mean of triplicate injections using a linear regression model. The results measured with the PFI-CL and AOAC Standard Methods, including the dilution factor are compared in Table 3. The good agreement between the PFI-CL and differential pulse polarography (DPP) official methods show the high degree of reliability of the PFI-CL method developed in this study. There was no significant difference (t-test) between the mean values obtained by both methods at 95% confidence.

The recovery of the PFI-CL method was obtained by spiking three amounts of each sulphite standard to diluted and homogenised pickled food sample solutions. The recovery was in the range 96.2–105.6%, as shown in Table 4, which means that no matrix effects are observed.

Table 2
Maximum tolerable concentration of coexistent substances for the determination of 2.0 mg L^{-1} sulphite.

Tolerance (mg L^{-1})	Coexistent substances
20,000	Cl^-, glucose, sucrose, ethanol, ascorbic acid
2000	CH_3COO^-, HPO_4^{2-}, Na^+, K^+, Mg^{2+}
200	NO_3^-, Ni^{2+}
20	SO_4^{2-}, Fe^{2+}, Co^{2+}
2	I^-, S^{2-}, Mn^{2+}, Fe^{3+}

Table 3
Comparative results for the determination of sulphite in different foods (mg kg^{-1} of sulphite).

Sample	PFI-CL method	AOAC method	Dilution factor
Sour bamboo shoots$^{0.57}$	1317.6 ± 5.8	1315.6 ± 2.1	1000
Pickled mustard greens$^{0.36}$	466.5 ± 1.8	467.1 ± 2.1	100
Bean spout$^{1.75}$	162.4 ± 3.8	154.4 ± 5.4	20
Pickled cabbage$^{0.87}$	110.8 ± 1.7	108.5 ± 4.2	20
Sultana raisins$^{0.16}$	92.9 ± 4.0	92.0 ± 6.5	10
Pickled eggplant$^{0.77}$	45.2 ± 1.0	44.3 ± 0.8	10
Pickled ginger$^{1.92}$	22.7 ± 0.3	25.6 ± 2.6	10
Pickled bamboo shoots$^{0.77}$	27.0 ± 0.4	26.1 ± 2.0	3
Sugar$^{1.69}$	25.3 ± 0.6	24.5 ± 1.4	5
Tapioca starch$^{0.84}$	18.4 ± 0.4	17.9 ± 0.7	5

All measurement was conducted in triplicate under optimal conditions (Table 1) ($t_{calculated}$ values are given by superscript letters and are less than $t_{critical}$ 2.776 at 95% confidence).

Table 4
Recovery of the PFI-CL method determined by analysing spiked dilute samples.

Sample	SO_3^{2-} present (mg kg^{-1})	SO_3^{2-} added (mg kg^{-1})	Found (mg kg^{-1})	Recovery
Sour bamboo shoots	1.58	1.5	3.11	101.7
		2.5	3.99	96.2
		3.5	4.98	97
Pickled mustard greens	1.56	1.5	3.09	101.9
		2.5	3.99	97.3
		3.5	5.05	99.6
Pickled ginger	1.76	1.5	3.35	105.6
		2.5	4.18	96.5
		3.5	5.17	97.3

4. Conclusions

The PFI-CL system outlined in the present paper allows fast, inexpensive, sensitive and selective determination of sulphite in food samples. Due to its simplicity and cost effectiveness, acidic $KMnO_4$ was chosen as the chemiluminescence reagent for this investigation. An on-line method for separation of sulphite from food matrices and interferences was successfully developed. The sensitivity of chemiluminescence detection system also was enhanced by using rhodamine B as a sensitiser for an acidic potassium permanganate reaction in the presence of polyphosphate. When the real samples are analysed directly by PFI-CL, the results obtained were close to those given by a standard AOAC method for sulphite determination. The performance of the developed method is better than existing DPP standard procedure, which still requires a tedious and time consuming acid distillation procedure for real sample preparation. Additional research has shown that the described system *unfortunately* fails for wines and alcoholic beverages because at very high ethanol concentration the CL emission intensity is suppressed seriously; however, this paper demonstrates that the described system can be used easily for both fresh and pickled food analysis.

Acknowledgements

The authors wish to thank the Thailand Research Fund (TRF) for financial support and Dr. T.J. Cardwell (La Trobe University, Australia) for providing the pervaporation unit. Additionally, S. Liawruangrath would like to express her sincere thanks and appreciation to the Centre for Innovation in Chemistry, Postgraduate Education and Research Program in Chemistry (PERCH-CIC), the commission on Higher Education for their partial support.

References

Association of Official Analytical Chemists (1990). *Official methods of analysis: Food composition, additives, natural contaminants* (15th ed.). Virginia: AOAC.

Adcock, J. L., Francis, P. S., & Barnett, N. W. (2007). Acidic potassium permanganate as a chemiluminescence reagent – A review. *Analytica Chimica Acta, 601*, 36–67.

Adcock, J. L., Francis, P. S., & Barnett, N. W. (2009). Chemiluminescence spectra for the oxidation of sulphite in the presence of fluorescent and non-fluorescent enhancers. *Analytica Chimica Acta, 652*, 303–307.

Al-Tamrah, S. A., Townshend, A., & Wheatley, A. R. (1987). Flow injection chemiluminescence determination of sulphite. *Analyst, 112*, 883–886.

Amador-Hernandez, J., & Luque de Castro, M. D. (2000). Pervaporation: A useful tool in food analysis. *Food Chemistry, 68*, 387–394.

Azevedo, C. M. N., Araki, K., Toma, H. E., & Angnes, L. (1999). Determination of sulfur dioxide in wines by gas-diffusion flow injection analysis utilizing modified electrodes with electrostatically assembled films of tetraruthenated porphyrin. *Analytica Chimica Acta, 387*, 175–180.

Chinvongamorn, C., Pinwattana, K., Praphairaksit, N., Imato, T., & Chailapakul, O. (2008). Amperometric determination of sulphite by gas diffusion-sequential injection with boron-doped diamond electrode. *Sensors, 8*, 1846–1857.

Food Standards Agency, Annual Report 2005/2006. EC Directive 89/2003 [online]. <http://www.food.gov.uk/multimedia/pdfs/annualreport0506.pdf>.

George, B. J., Pereira, N., Al Massum, M., Kolev, S. D., & Ashokkumar, M. (2008). Sensitivity enhancement in membrane separation flow injection analysis by ultrasound. *Ultrasonics Sonochemistry, 15*, 151–156.

Gonzalez-Rodroguez, J., Perez-Juan, P., & Luque de Castro, M. D. (2002a). Method for monitoring urea and ammonia in wine and must by flow injection–pervaporation. *Analytica Chimica Acta, 471*, 105–111.

Gonzalez-Rodroguez, J., Perez-Juan, P., & Luque de Castro, M. D. (2002b). Sequential spectrophotometric determination of methanol and iron in vinegar by a flow injection–pervaporation method. *Analytical and Bioanalytical Chemistry, 374*, 120–125.

Hassan, S. S. M., Hamza, M. S. A., & Mohamed, A. H. K. (2006). A novel spectrophotometric method for batch and flow injection determination of sulfite in beverages. *Analytica Chimica Acta, 570*, 232–239.

Hanssen, M., Marsden, J., & Norris, B. (1989). *The new additive code breaker*. Melbourne: Lothian Publishing. p. 47.

Hindson, B. J., & Barnett, N. W. (2001). Analytical applications of acidic potassium permanganate as a chemiluminescence reagent. *Analytica Chimica Acta, 445*, 1–19.

Huang, Y., Zhang, C., Zhang, X., & Zhang, Z. (1999). Chemiluminescence of sulfite based on auto-oxidation sensitized by rhodamine 6G. *Analytica Chimica Acta, 391*, 95–100.

Izquierdo-Ferrero, J. M., Romero, J. M. F., & Luque de Castro, M. D. (1997). On-line flow injection–pervaporation of beer samples for the determination of diacetyl. *Analyst, 122*, 119–122.

Lin, J., & Hobo, T. (1996). Flow-injection analysis with chemiluminescent detection of sulphite using Na_2CO_3–$NaHCO_3$–Cu^{2+} system. *Analytica Chimica Acta, 323*, 69–74.

Luque de Castro, M. D., & Papaefstathiou, I. (1998). Analytical pervaporation: A new separation technique. *TrAC Trends in Analytical Chemistry, 17*, 41–49.

Mataix, E., & Luque de Castro, M. D. (1998). Determination of total and free sulfur dioxide in wine by pervaporation–flow injection. *Analyst, 123*, 1547–1549.

Meste, Y. F., Zamora, L. L., & Calatayud, J. M. (2001). Flow-chemiluminescence: A growing modality of pharmaceutical analysis. *Luminescence, 15*, 213–235.

Nacapricha, D., Sangkarn, P., Karuwan, C., Mantim, T., Waiyawat, W., Wilairat, P., et al. (2007). Pervaporation–flow injection with chemiluminescence detection for determination of iodide in multivitamin tablets. *Talanta, 72*, 626–633.

Papaefstathiou, I., Luque de Castro, M. D., & Valcarcel, M. (1996). Flow-injection/pervaporation coupling for the determination of sulphide in Kraft liquors. *Fresenius' Journal of Analytical Chemistry, 354*, 442–446.

Ruiz-Capillas, C., & Jiménez-Colmenero, F. (2009). Application of flow injection analysis for determining sulphites in food and beverages: A review. *Food Chemistry, 112*, 487–493.

Satienperakul, S., Sheikheldin, S. Y., Cardwell, T. J., Cattrall, R. W., Luque de Castro, M. D., & Kolev, S. D. (2003). Pervaporation–flow injection analysis of phenol after on-line derivatisation to phenyl acetate. *Analytica Chimica Acta, 485*, 37–42.

Su, X., & Wie, W. (1998). Flow injection determination of sulphite in wines and fruit juices by using a bulk acoustic wave impedance sensor coupled to a membrane separation technique. *Analyst, 123*, 221–224.

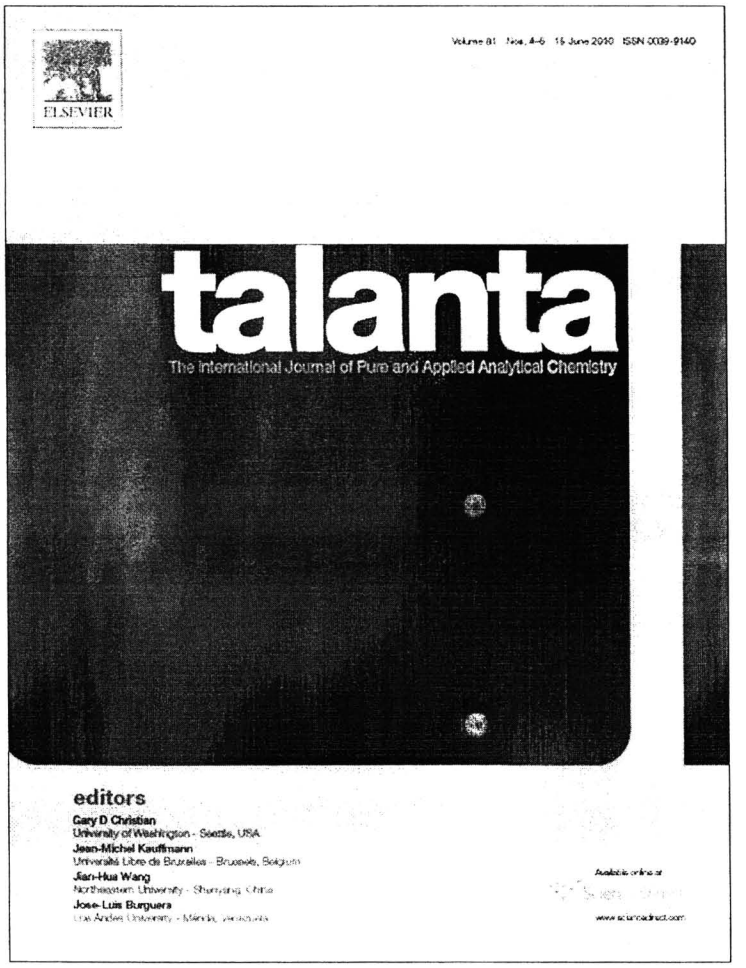
Sulistyarti, H., Cardwell, T. J., Luque de Castro, M. D., & Kolev, S. D. (1999). On-line determination of cyanide in the presence of sulfide by flow injection with pervaporation. *Analytica Chimica Acta, 390*, 133–139.

Tavares Araujo, C. S., Lira de Carvalho, J., Ribeiro Mota, D., Araujo, C. L., & Coelho, N. M. M. (2005). Determination of sulphite and acetic acid in foods by gas permeation flow injection analysis. *Food Chemistry, 92*, 765–770.

Wang, L. J., Cardwell, T. J., Cattrall, R. W., Luque de Castro, M. D., & Kolev, S. D. (2003). Determination of ammonia in beers by pervaporation flow injection analysis and spectrophotometric detection. *Talanta, 60*, 1269.

Warner, C. R., Diachenko, G. W., & Bailey, C. J. (2000). *Sulfites: An important food safety issue, food testing and analysis*. US Food and Drug Administration, Target Group, September 2000 [online]. <http://www.cfsan.fda.gov/~dms/fssulfit.html>.

Yamada, M., Nakada, T., & Suzuki, S. (1983). The determination of sulphite in a flow injection system with chemiluminescence detection. *Analytica Chimica Acta, 147*, 401–404.



Talanta 81 (2010) 1793–1799

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of sulfite by pervaporation-flow injection with amperometric detection using copper hexacyanoferrate-carbon nanotube modified carbon paste electrode

Lori Shayne T. Alamo[a,b], Tanin Tangkuaram[a], Sakchai Satienperakul[a,*]

[a] Department of Chemistry, Faculty of Science, Maejo University, Sansai, Chiang Mai 50290, Thailand
[b] Department of Chemistry, College of Arts and Sciences, Nueva Vizcaya State University, Bayombong, Nueva Vizcaya 3700, Philippines

ARTICLE INFO

Article history:
Received 7 January 2010
Received in revised form 19 March 2010
Accepted 22 March 2010
Available online 27 March 2010

Keywords:
Sulfite
Flow injection
Pervaporation
Copper hexacyanoferrate-carbon nanotube modified carbon paste electrode

ABSTRACT

A pervaporation-flow injection (PFI) method was developed for the determination of sulfite in selected food samples using a copper hexacyanoferrate-carbon nanotube (CuHCF-CNT)-modified carbon paste electrode. The electrochemical behavior of the modified electrode was observed using cyclic voltammetry in comparison to a CuHCF-modified carbon paste electrode and a bare carbon paste electrode at a scan rate of 100 mV s^{-1} in 0.10 M KNO_3. The bare carbon paste electrode gave the lowest response to sulfite, while the presence of CuHCF made the detection of sulfite possible through electrocatalytic oxidation by the hexacyanoferrate in the modified electrodes. The presence of CNT in the CuHCF-CNT-modified sensor gave the most remarkable current for the detection of sulfite and was then used as a working electrode in the amperometric flow-through cell in the pervaporation flow injection system. The PFI method involves the injection of a standard or sample sulfite solution into a sulfuric acid donor stream to generate sulfur dioxide gas and evaporate into the headspace of the pervaporation unit. The sulfur dioxide diffuses through the PTFE hydrophobic membrane into a potassium nitrate acceptor stream and reverts to the sulfite form, which, subsequently, is transported to the electrochemical flow cell where it is analyzed amperometrically at a CuHCF-CNT-modified electrode at +0.55 V (vs. Ag/AgCl). The detection was determined to be applicable in the sulfite concentration range of 0.5–50 mg L^{-1}. The sensitivity, detection limit, and sample throughput were determined to be 2.105 nA L mg^{-1}, 0.40 mg L^{-1} and 11 h^{-1}, respectively. The developed PFI method, coupled with the CuHCF-CNT-modified carbon paste electrode, was applied in the determination of sulfite content in sulfite-containing food products. The results agreed well with those obtained through the officially recommended differential pulse polarographic method.

1. Introduction

Sulfites are used as preservatives to prevent oxidation, inhibit bacterial growth, and control enzymatic and non-enzymatic reactions with stabilizing and conditioning functions [1]. Despite these useful advantages, sulfite should be applied in strictly limited amounts due to its potential toxicity. Hence, products containing more than the established threshold sulfite level should be labeled adequately. Due to the allergic effect on hypersensitive individuals such as gastric irritation, nausea, diarrhea, nettle rash or swelling, and asthmatic attacks, sulfite detection has been a study of wide interest [2]. The existence of accurate methods for the determination of sulfites is necessary fundamentally for the food industry to ensure product quality. Previous methods available for determination of sulfites include iodometric titration [3], conductimetry [4], photometry [5,6], chemiluminescence [7], and capillary electrophoresis [8]. Most of these methods, however, need vast sample pre-treatment and solution preparation and result in low specificity and sensitivity in detection. Thus, simpler methods are currently and constantly being studied. Isaac et al. [9] presented an overview of the latest developments in electrochemical procedures for sulfite determination in food and beverages. Decnop-Weever and Kraak [10], Hassan et al. [11], and Tzanavaras et al. [12] have developed flow-spectrophotometric methods for the determination of sulfite in wines and other beverages. Dadamos and Teixeira developed an electrochemical sensor responsible for the electrocatalytic oxidation of sulfite on a platinum electrode modified with nanostructured copper salen (salen = *N,N′*-ethylene*bis*(salicylideneiminato)) polymer films [13]. Zhou et al. developed an amperometric sensor based on multi-walled carbon nanotubes/ferrocene-branched chitosan composites

* Corresponding author. Tel.: +66 53 873530; fax: +66 53 878225.
E-mail address: sakchais@mju.ac.th (S. Satienperakul).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.03.043

for the determination of sulfite [14]. Lucero et al. studied the electrocatalytic oxidation of sulfite using a polymeric iron tetra (4-aminophenyl) porphyrin-modified electrode [15]. These developments of sensors for the determination of sulfite all have been of remarkable interest due to a number of advantages brought about by sensors application such as rapid response, high specificity and sensitivity, low cost, and the elimination of sample preparation.

Since their discovery in 1991, carbon nanotubes (CNT) have been of great interest in research; and their various applications have continuously been under study. According to the recent review by Agüí et al., CNT have received great attention for the preparation of electrochemical sensors and biosensors, which based on carbon nanotubes-driven electrocatalytic effects. The construction and analytical usefulness of new hybrid materials with polymers or other nanomaterials were widely reported [16]. Wang et al. previously used multi-walled carbon nanotubes (MWCNT) in the construction of a working electrode by mixing MWCNT with copper powder and mineral oil for use as a capillary electrophoresis detector in carbohydrate determination [17]. Jia et al. developed a new method of constructing a needle-type biosensor based on carbon nanotubes for the detection of glucose [18]. A mixture of MWCNT, graphite powder and glucose oxidase freeze-dried powder was packed into a glass capillary with an inner diameter of 0.5 mm. The resulting biosensor was electrochemically characterized through amperometry–a method that is based on the measurement of electric current at a constant operating potential. The chemical properties of carbon nanotubes, such as enhanced electrical conductivity, chemical inertness, stability, and their capability to promote electron transfer reactions as an electrode with electroactive species in solution, make CNT even more interesting in the application of electrochemical analysis [19,20].

Metal hexacyanoferrates (MHCF) have been a study of wide interest due to their electroactive properties as excellent electron transfer mediators [21]. MHCF have been fabricated using various transition metal cations such as iron [22,23], cobalt [24,25], tin [26], indium [27], silver [28], zinc [29], chromium [30,31], and copper [32–34]. Copper (II) hexacyanoferrate (CuHCF), have been previously investigated to be a good mediator in the catalytic oxidation of sulfite [35], has been used in this study particularly due to the CuHCF mediator's ease of preparation as well as integration into modified carbon paste working electrodes for the determination of sulfite. Ravi Shankaran and Sriman Narayana developed the method utilizing a CuHCF-modified graphite electrode for the amperometric determination of sulfite [35]. Nevertheless, a CuHCF-CNT carbon paste electrode has never been utilized for sulfite determination.

Flow injection analysis of sulfite, coupled with electrochemical detection, has become a method of choice due to the direct electrochemical oxidation of sulfite [22,36–37]. The development of sensors for the determination of sulfite is of remarkable interest due to a number of advantages such as rapid response, high specificity and sensitivity, low cost, and the elimination of sample preparation when it is used with an effective on-line separation technique such as pervaporation [38,39].

In this study, an on-line pervaporation-flow injection (PFI) method, using a highly sensitive CuHCF-CNT electrochemical sensor, was developed for the determination of sulfite in food products where the different aggregation and physical properties of the sample matrices always are a major problem and require sophisticated sample pre-treatment prior to analysis. A pervaporation unit incorporated in the flow system was expected to improve the selectivity while the CuHCF-CNT-modified electrode in an amperometric flow-through cell enhanced the sensitivity of the flow injection system.

2. Experimental

2.1. Reagents and chemicals

All reagents were of analytical grade, and all solutions were prepared using deionized water (Millipore, France). The supporting electrolyte used for the cyclic voltammetry and amperometric determination was 0.1 M KNO_3 (Sigma, USA). The pH of the 0.1 M KNO_3 acceptor stream for the PFI analysis was adjusted using 2.0 M NaOH (Labscan, Ireland) or 2.0 M HCl (Ajax, Australia) solutions where necessary. The 0.050 M H_2SO_4 donor solution was prepared by dissolving the appropriate amounts of concentrated H_2SO_4 (Merck, Germany) in deionized water. Both the donor and acceptor solutions were subjected to ultrasonication before use. A sulfite stock solution (1000 mg L^{-1}) was prepared by dissolving 0.1589 g of anhydrous Na_2SO_3 (J.T. Baker, USA) in 100 ml of deionized water and standardizing by titrating with iodine. This stock solution was kept in a sealed container in a refrigerator at 4 °C when not in use. A series of standard solutions (0.5–50 mg L^{-1}) were prepared using the appropriate dilution of the stock sulfite solution in deionized water.

2.2. Electrode construction

The CuHCF-CNT-modified working electrode was prepared by mixing various ratio compositions of multi-walled carbon nanotube (CNT) (30 ± 15 nm OD, >95% purity, NanoLab Inc., USA), graphite powder (≤20 μm in particle size, synthetic, Aldrich, USA) and mineral oil (Sigma, USA) in an agate mortar. The impure CNT used was previously purified by immersing the tubes in concentrated HNO_3 (Merck, Germany) and subjecting it to ultrasonication for 12 h [35]. The CuHCF mediator was prepared previously by mixing equal volumes of 0.2 M $Cu(NO_3)_2$ (Ajax, Australia) and 0.1 M $K_4Fe(CN)_6$ (Fisher Scientific, UK), heating for an hour in a water bath until the solution dried, and obtaining only the brown CuHCF solid as described previously with slight modification [34]. A portion of each of the resulting carbon pastes was then packed firmly into the cavity of a 3.0 mm diameter Teflon tube (Metrohm, Switzerland) for individual analysis with a stainless steel screw serving as the electrical contact. The resulting electrode surfaces were smoothed using an oil-removing film before examination.

2.3. Apparatus

Cyclic voltammetric and amperometric experiments were carried out using a potentiostat (NSTDA Glucosen Electrochemical Analyzer, Thailand, and CHI1230A CH Instrument, TX, USA). A three electrode system was used with a Platinum wire auxiliary/counter electrode (Sigma, USA), an Ag/AgCl reference electrode (3 M KCl) (CHI111 CH Instrument, TX, USA), and one of the modified carbon paste electrodes as the working electrode.

The amperometric PFI system utilized one two-channeled and one four-channeled peristaltic pump with rate selectors (Gilson, France), one six-port injection valve (Upchurch Scientific, USA), PTFE tubing (TACS, Australia) with an internal diameter of 0.5 mm, and a homemade Perspex pervaporation unit. A flow-through amperometric measuring cell, contained in a Faraday Cage (Autolab, Netherlands), was used for the detection in which the modified carbon paste working electrode was incorporated along with an Ag/AgCl reference electrode (3 M KCl), and a gold counter electrode. Potentials were applied using a potentiostat (791 VA Metrohm, Switzerland) and the detector output was recorded using a portable computer (IBM, Mexico) connected via a USB-RS232 serial port of a digital multimeter (Uni-Trend, Hong Kong).

The pervaporation unit composed of hexagonal donor and acceptor chambers with 0.3 and 5 mm depths, respectively, similar

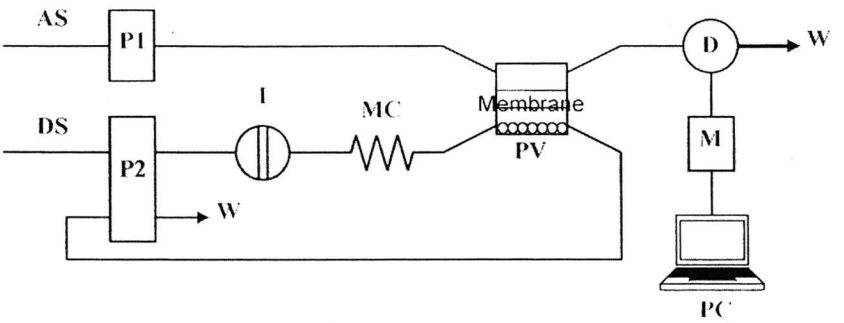


Fig. 1. Schematic diagram of the PFI system used: AS = acceptor solution (0.10 M KNO_3, 2.0 mL min $^{-1}$); DS = donor solution (0.050 M H_2SO_4, 0.75 mL min^{-1}); P = peristaltic pump; I = sample injection port (300 μL sample injection volume); MC = mixing coil (100 cm); PV = pervaporation unit; D = amperometric flow cell; M = digital multimeter; PC = portable computer; and W = waste.

to that described previously [40]. The donor chamber was packed with a single layer of 3 mm-diameter glass beads for an improved reproducibility and sample throughput [41]. A PTFE membrane (Trace Biotech, Germany) with a thickness of 1.5 mm and a diameter of 4.0 cm was positioned to separate the donor and acceptor chambers to prevent direct contact with the suspended food sample.

2.4. Procedure

The cyclic voltammetric measurements were investigated at a potential range of −0.5 to 1.5 V at 100 mV s^{-1} using the bare, the CuHCF-modified, and the CuHCF-CNT-modified carbon paste working electrodes with 0:80:0:20, 0:70:10:20, and 10:60:10:20 weight compositions of CNT, graphite, CuHCF, and mineral oil, respectively. With the Glucose potentiostat, amperometric measurements of sulfite were carried out at multiple potentials set at 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 V to obtain the current–voltage curves of the modified carbon paste electrodes. A series of 1.2 mM additions of sulfite were injected into the measuring cell containing 2.0 mL of supporting electrolyte and the respective currents were measured amperometrically.

For the PFI analysis, physical and chemical parameters were varied and optimized to obtain optimum results for sulfite detection. The working electrode composition was varied from 0% to 60% CNT, with fixed CuHCF and mineral oil amounts of 10% and 20%, respectively. The amount of graphite used depended inversely on CNT composition in order to retain the correct percentages in the carbon paste.

A 300 μL sample or standard sulfite solution is injected manually via an injection valve into a 0.050 M H_2SO_4 donor stream with the flow rate of 0.75 mL min^{-1} controlled by the first peristaltic pump. The resulting mixture of sulfite solution and the sulfuric donor stream is transported to the donor chamber of the pervaporation unit where it is converted to sulfur dioxide and evaporates into the headspace. Sulfur dioxide gas then diffuses across the PTFE membrane into an acceptor solution containing a 0.10 M sodium nitrate solution. Sulfur dioxide hydrolyses in the acceptor solution and then reverts into the sulfite form, which is transported subsequently to the electrochemical flow cell where it is analyzed amperometrically at a CuHCF-CNT-modified working electrode at +0.55 V (vs. Ag/AgCl). The second peristaltic pump is employed to both propel and withdraw the donor solution from the pervaporation unit; it also assists in the maintenance of a constant liquid

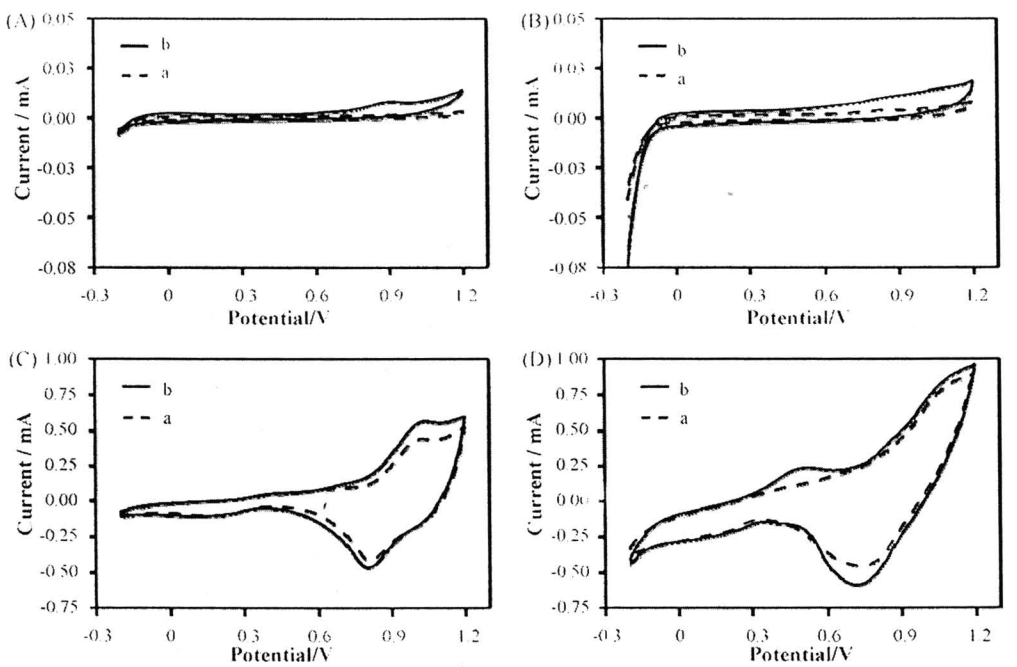


Fig. 2. Cyclic voltammograms for (a) blank and (b) 1.2 mM Na_2SO_3 at (A) bare carbon paste electrode (CPE), (B) CNT-CPE, (C) CuHCF-CPE, and (D) CuHCF-CNT modified electrodes. Supporting electrolyte: 0.1 M KNO_3; scan rate 100 mV s $^{-1}$.

level in the donor chamber where the glass beads are submerged completely [41].

At the obtained optimized conditions, amperometric PFI measurements were conducted following the scheme shown in Fig. 1. All measurements were carried out at room temperature.

The Association of Official Analytical Chemists' (AOAC) standard differential pulse polarographic method (987.04) [42] was performed using a voltammograph (Metrohm model VA 741, Metrohm Ltd. Switzerland). All samples were acid distillated by purging with oxygen-free nitrogen and collected in an electrolyte-trapping solution (2 M ammonium acetate buffer with 5% ethanol) prior to polarographic analysis.

3. Results and discussion

3.1. Cyclic voltammetric and amperometric measurements

The electrochemical behavior of sulfite towards the proposed CuHCF-CNT modified carbon paste electrode was initially investigated through cyclic voltammetry. Fig. 2 shows the cyclic voltammograms of the four modified working electrodes using a potentiostat. As observed in Fig. 2, voltammograms A (a and b), the magnitude of current detected by the bare electrode was very small with slightly visible response to sulfite oxidation upon addition at about +0.90 V. On the other hand, curve B (a) corresponds to the cyclic voltammogram of the CNT modified electrode in the supporting electrolyte, and curve B (b) with the presence of 1.2 mM sulfite solution. No significant change in the sulfite oxidation peak was observed, indicating that CNT has no effect upon the sulfite catalytic behavior. The CuHCF-modified electrode yields a substantially higher oxidative current over bare and CNT electrodes (notice the different current scales A, B vs. C), and indicates the catalytic oxidation of sulfite by the CuHCF substance. The CuHCF modifier is essential in the catalytic electrochemical oxidation of sulfite, which takes place at the surface of the electrode. Ferricyanide, which is electrochemically generated and present at the electrode surface, chemically oxidizes the sulfite present in the solution into sulfate:

$$Fe(II)(CN)_6^{4-} \rightarrow Fe(III)(CN)_6^{3-} + e^- \quad (1)$$

$$2Fe(III)(CN)_6^{3-} + SO_3^{2-} + H_2O \rightarrow 2Fe(II)(CN)_6^{4-} + SO_4^{2-} + 2H^+ \quad (2)$$

While this happens, the electrochemically generated ferricyanide is then reduced to ferrocyanide, which later again becomes electrochemically oxidized [35].

Fig. 2 curves D (a and b) correspond to the cyclic voltammograms obtained using the CuHCF-CNT modified electrode. Even more enhanced anodic currents were achieved using the CuHCF-CNT-modified electrode in blank 0.1 M KNO_3 supporting electrolyte (D (a)) and in the presence of 1.2 mM sulfite (D (b)), respectively, the latter indicating that sulfite was catalytically oxidized by the electrode (the observed oxidative peak at ±0.48 V).

Fig. 3 corresponds to the current–voltage curves obtained for the (a) bare carbon paste, (b) CNT, (c) CuHCF, and (d) CuHCF-CNT modified electrodes in the presence of 1.2 mM sulfite. A series of 1.2 mM sulfite samples were placed into an electrochemical cell and amperometric measurements were taken at 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 V working potentials. A significantly enhanced peak for the CuHCF-CNT-modified electrode may be due to the higher active surface area upon the deposition of CNT into the modified electrode [43] and its unique conductivity properties of CNT [44] made faster electron transfer between CNT and CuHCF.

In addition, a voltage peak shift to the left of the potential axis implies that CNT are more suitable for the detection of sulfite since sulfite is more easily oxidized in the CuHCF-modified electrode presence of CNT.

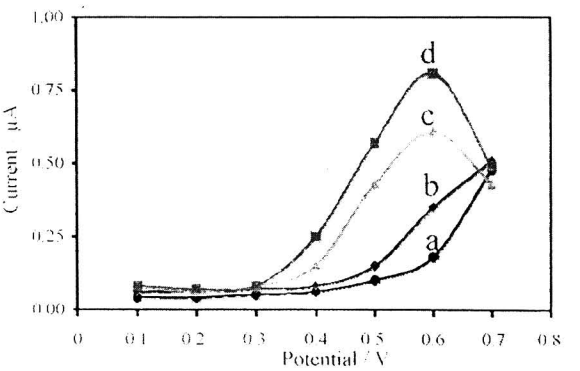


Fig. 3. Current–voltage curves for 1.2 mM Na_2SO_3 in 0.10 M KNO_3 at a (a) bare carbon paste electrode, (b) CNT, (c) CuHCF, and (d) CuHCF-CNT modified electrodes.

3.2. Pervaporation-flow injection (PFI) analysis

After studying its electrochemical behavior, the CuHCF-CNT-modified carbon paste electrode was applied to the amperometric PFI analysis of sulfite. PFI system parameters that affect the sensitivity of sulfite determination were studied. Table 1 lists these parameters including the range over which each parameter was studied and the optimum conditions. The electrode composition of the CuHCF-CNT-modified sensor was optimized by varying the carbon nanotube composition from 0% to 50% (reduce the % graphite from 50 to 0, respectively) and keep the percentage of CuHCF and oil constant. The peak height was measured as the sensor detected a fixed amount of sulfite injected into the system. The current produced with the modified carbon paste electrode without CNT was almost equal to the modified electrodes containing 10 and 20% CNT. The maximum current signal was obtained with the modified electrode containing 30–40% CNT and it keeps plateau until 50% CNT. At these CNT compositions the background current were also high, however, the 35% CNT was giving the highest sensitivity as illustrated by the maximum signal-to-noise ratio. Therefore, the CuCHF-CNT-modified carbon paste electrode was determined to be optimum at an electrode composition of 35% CNT, 35% graphite, 10% CuHCF, and 20% mineral oil.

The criteria for the selection of the optimized parameters include low and stable background current during analysis, and a well-defined peak shape for the detection of sulfite. Best results were obtained in a background acceptor electrolyte which is composed of 0.10 M KNO_3.

The generation of SO_2 gas takes place in an acidic medium and in most gas diffusion-flow injection sulfite determinations; the solution of sulphuric acid is commonly used. The evaluation of the H_2SO_4 concentration, used as a donor stream solution, was carried out over the range 0.025–0.10 M with the flow rate at 0.5 mL min^{-1}. The maximum current was obtained at the concentration of 0.050 M and was considered to be optimal using the univariate approach.

The optimum potential for amperometric detection in pervaporation-flow injection (PFI) analysis was obtained by studying the hydrodynamic voltammetric behavior of sulfite towards the CuHCF-CNT-modified carbon paste electrode. Initially, the range over which the working potential was studied from 0.10 to 1.00 V. However, at very low potentials, very low to zero detection occurred. On the contrary, at very high working potentials, extremely high background noise was observed, hence, lowering the sensitivity of the detection as determined by the signal-to-noise ratio of the injected sulfite standard. Thus, the

Table 1
Pervaporation-flow injection system parameters optimized in this study.

Parameters	Range studied	Optimal value
Acceptor stream composition	0.10 M phosphate buffer (pH 7.0, 7.4); 0.10 M phosphate buffer (pH 8.0)/0.1% SDS; 0.10 M phosphate buffer (pH 7.4)/0.1 M NH_4Cl; 0.10 M phosphate buffer (pH 7.4)/0.01 M Na_2SO_4; 0.10 M NH_4Cl; 0.10 M KCl; 0.10 M KNO_3; 0.10 M KNO_3 and 0.05 M NaOH; 0.10 M KNO_3 and 0.05 M $NaHCO_3$; 0.10 M KNO_3 and 0.01 M NaOH; 0.10 M KNO_3 and 0.01 M $NaHCO_3$	0.10 M KNO_3
Sulfuric acid donor stream concentration (M)	0.025–0.100	0.050
Working electrode composition* (percent CNT by weight)	0–50	35
Electrode potential (V)	0.10–1.00	+0.55
Donor stream flow rate (mL min^{-1})	0.5–2.5	0.75
Acceptor stream flow rate (mL min^{-1})	0.5–3.0	2.0
Sample injection volume (μL)	50–500	300

* The amount of CuHCF and mineral oil added for the construction of the modified working electrode was fixed at 10% and 20% by weight, respectively. The amount of graphite added depended inversely on the amount of CNT used in order to achieve the 100% sample by weight.

working potential range from 0.50 to 0.60 V was more closely evaluated with respect to the peak currents of the injected sulfite standard and the corresponding background currents. The working potential yielded a maximum sensitivity at +0.55 V (versus Ag/AgCl).

The effect of the flow rates of the donor and acceptor streams were studied separately. As expected, the sensitivity was higher at a lower donor stream flow rate but the sample throughput obtained was lower as well. A flow rate of 0.75 mL min^{-1} was then selected to compromise for both the sensitivity and the sample throughput.

As for the acceptor stream flow rate, sample throughput also increased as the rate of flow was increased. The signal detection, however, was highest at a rate of 2.0 mL min^{-1}, and thus, was selected for the acceptor stream flow rate.

Similar to the flow rate, the sample injection volume also affected sensitivity and sample throughput but in the opposite manner. A sample injection volume of 300 μL was selected in resolution to the sensitivity and sample throughput requirements.

3.3. Analytical figures of merit

At the optimum working conditions, the calibration curve for sulfite determination was obtained to be linear (R^2 = 0.9987) in the concentration range of 0.5–50.0 mg L^{-1}. The linear regression equation in this range can be expressed as $I = 2.105\,C + 5.227$, giving a sensitivity of 2.105 nA mg^{-1} L. A further increase in concentration of the sulfite standards starts to cause a deflection to linearity of the signal with respect to concentration. The limit of detection (LOD), determined experimentally as the lowest sulfite concentration that gives a current signal of three times the background noise, was 0.40 mg L^{-1}. The sample throughput was determined to be 11 h^{-1}.

The compactness of CNT-CuHCF electrode surface is desirable, since it allows the laminar flow in the wall- jet type amperometric flow cell and is detrimental to the reproducibility and lifetime of the sensor. Thus, the sensor is preferably used after being prepared within a day and kept away from open air. The analytical signals corresponding to peak minima were found to be reproducible up to 120 injections (RDS <5%), for the determining of 25.0 mg L^{-1} Na_2SO_3.

In addition, the calibration plot obtained for the determination of sulfite using the developed CuHCF-CNT-modified working electrode was compared to the calibration responses to sulfite using a simple CuHCF-modified carbon paste electrode and a bare glassy carbon electrode as employed in pervaporation-flow injection analysis (Fig. 4). The sensitivity of the developed CuHCF-CNT-modified carbon paste working electrode was the highest at 2.105 nA mg^{-1} L (R^2 = 0.9987) compared to the sensitivities of CuHCF-modified carbon paste and bare carbon paste electrodes of 1.043 nA mg^{-1} L (R^2 = 0.9829) and 0.029 nA mg^{-1} L (R^2 = 0.9833) while the LODs for the other two electrodes were found to be 1.5 mg L^{-1} and 10 mg L^{-1}, respectively.

Table 2
Tolerance limits of possible interferences in the PFI method.

Tolerance ratio*	Interferences
1:100	Na^+, K^+, Ca^{2+}, Zn^{2+}, Cl^-, CH_3COO^-
1:10	Mg^{2+}, Ni^{2+}, SO_4^{2-}, HPO_4^{2-}, sucrose
1:1	Fe^{3+}, Mn^{2+}, Co^{2+}, ascorbic acid
1:<1	I^-

* The tolerance ratio indicates how much equivalent interference (I) concentration can be tolerated per 25.0 mg L^{-1} Na_2SO_3 control (C) in this manner: C: I.

3.4. Interference study

Possible interferences of food matrices to the PFI method were studied. The tolerance ratios of possible coexisting ions and compounds are listed in Table 2. The values were determined using 25.0 mg L^{-1} Na_2SO_3 as the control and tolerance was based on whether the interference causes an error of greater than 5%. Table 2 indicates that up to 100 times the Na_2SO_3 control can be tolerated for interferences such as sodium and chlorine; however, only equal amounts of other interferences such as iron and ascorbic acid can be tolerated and even less of iodine. The results can be attributed to the unique chemical property of the carbon nanotubes, its enhanced electrical conductivity, making it highly sensitive to the presence of ions which also possess redox properties, if such species could

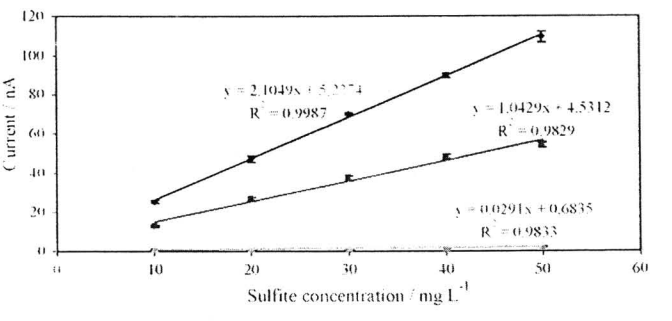


Fig. 4. Calibration curves of sulfite with (-◊-) CuHCF-CNT, (-●-) CuHCF, and (-Δ-) bare carbon paste working electrodes.

Table 3
Comparison of results obtained by PFI and DPP methods for sulfite in pickled food.

Sample	Sulfite content (mg kg^{-1})		Percentage recovery for proposed PFI method
	PFI method	DPP method	
Bean spout	162 ± 3	154 ± 5	96.8–104.6
Pickled cabbage	110 ± 1	102 ± 4	97.5–102.9
Pickled bamboo shoot	27.0 ± 0.4	26 ± 2	96.2–101.9
Pickled ginger	23.1 ± 0.5	25 ± 3	97.5–104.8

Standard deviation calculated on the basis of three replicate measurements.

reach the electrode surface. However, these species were found to suppress the analytical signals as the concentration ratios were increased. This can be explained by the redox character of these species resulting in lower production of volatile SO_2 in the donor chamber, particularly with the I^- interference. Higher concentrations of the interfering species in the standard solution may also hinder sulfite from evaporating into sulfur dioxide gas, hence, hindering the detection of the genuine concentration of the analyte by the PFI system. Therefore, sulfite standard solutions containing higher concentrations of the interfering ions caused significant interference affecting the sulfite detection.

3.5. Real sample analysis

The developed PFI method was applied to the determination of sulfite in pickled food samples in order to evaluate the accuracy of the proposed PFI system. The sulfite concentration present in each pickled food sample was measured by standard addition method. A 50 g of each sample was weighed and was homogenized with an equivalent volume of deionized water. The resulting homogenized mixture was collected and served as the sample stock solution. A series of increasing concentrations from 5.0 to 25.0 mg L^{-1} of standard sulfite solution were next prepared containing equivalent amount of the sample stock solution and was diluted to mark with the donor solution (0.025 M H_2SO_4 solution). These prepared solutions were consecutively injected into the PFI system for analyzes.

The results were then compared with those obtained using differential pulse polarography (DPP), which is the standard method for sulfite determination as proposed by the Association of Official Analytical Chemists (AOAC) [42]. The results obtained by both methods, including the percentage recovery upon addition of certain amounts of standard sulfite solution, are shown in Table 3. The relative standard deviations for the analysis of sulfite in pickled food samples using the proposed PFI method were in the range of 1.0–2.2%.

The Student's *t*-test was also calculated for both methods at 98% confidence [45]. After comparing the calculated *t* values of each food sample obtained from PFI with the AOAC method, results revealed that the data obtained using the developed PFI method with the CuHCF-CNT-modified working electrode was reliable.

4. Conclusions

The presence of CuHCF made the detection of sulfite possible through the electrocatalytic oxidation of sulfite by hexacyanoferrate in the modified electrode. Moreover, the presence of CNT in the modified sensor gave an even more remarkable effect for the detection of sulfite. This promising result led to the fabrication of an amperometric flow detector for sulfite in a flow system.

CuHCF-CNT modified electrode is more effective in the determination of sulfite in aqueous solution as compared to the use of bare carbon paste or a CuHCF-modified carbon paste electrode. The developed electrode was successfully applied to pervaporation-flow injection analysis as an effective sensor for sulfite determination, allowing a higher selectivity during sulfite analysis and producing a good sensitivity and a relatively low detection limit. Using versatile designs of the pervaporation unit, food samples containing many matrices can be analyzed more selectively. Compared with the method based on photometric PFI proposed by Mataix and Luque de Castro [38] the current proposed method requires a less-complicated set-up and provides a wider linear range and lower detection limit. The proposed PFI method is also applicable in the analysis of real pickled food samples obtaining reliable results as compared to the standard method of sulfite determination.

Acknowledgments

L.S.T. Alamo would like to thank Assoc. Prof. Dr. Thep Phongparnich, the President of Maejo University, Chiang Mai, Thailand, for his invaluable support. S. Satienperakul would like to express his sincere thanks and appreciation to the Thailand Research Fund (TRF) for their partial support. The authors would like to thank Mr. Chatchai Ponchio from the Department of Chemistry, Faculty of Science, Rajamangala University of Technology Thanyaburi, for his kind support with the Glucosen instrument and Dr. T.J. Cardwell (La Trobe University, Australia) for providing the pervaporation unit.

References

[1] R. Walker, Food Addit. Contam. 2 (1985) 5.
[2] M. Hanssen, The New Additive Code Breaker, Lothian, Melbourne, 1989, 201p.
[3] American Public Health Association, Standard Methods For The Examination Of Water And Wastewater, 19th Edn., APHA, Washington DC, 1995, 4–131.
[4] S. McLeod, D.E. Davey, Anal. Chim. Acta 600 (2007) 72.
[5] A. Afkhami, N. Sarlak, Sens. Actuators B 124 (2007) 285.
[6] Y. Li, M. Zhao, Food Control 17 (2006) 975.
[7] H. Meng, F. Wu, Z. He, Y. Zeng, Talanta 48 (1999) 571.
[8] G. Jankovskiene, Z. Daunoravicius, A. Padarauskas, J. Chromatogr. A 934 (2001) 67.
[9] A. Isaac, J. Davis, C. Livingstone, A.J. Wain, R.G. Compton, TrAC Trend. Anal. Chem. 25 (2006) 589.
[10] L.G. Deenop-Weever, J.C. Kraak, Anal. Chim. Acta 337 (1997) 125.
[11] S.S.M. Hassan, M.S.A. Hamza, A.H.K. Mohamed, Anal. Chim. Acta 570 (2006) 232.
[12] P.D. Tzanavaras, E. Thiakouli, D.G. Themelis, Talanta 77 (2009) 1614.
[13] T.R.L. Dadamos, M.F.S. Teixeira, Electrochim. Acta 54 (2009) 4552.
[14] H. Zhou, W. Yang, C. Sun, Talanta 77 (2008) 366.
[15] M. Lucero, G. Ramirez, A. Riquelme, I. Azocar, M. Isaacs, F. Armijo, J.E. Forster, E. Trollund, M.J. Aguirre, D. Lexa, J. Mol. Catal. A 221 (2004) 71.
[16] L. Agüí, P. Yáñez-Sedeño, J.M. Pingarrón, Anal. Chim. Acta 622 (2008) 11.
[17] J. Wang, G. Chen, M. Wang, M.P. Chatrathi, Analyst 6 (2004) 512.
[18] J. Jia, W. Guan, M. Sim, Y. Li, H. Li, Sensors 8 (2008) 1712.
[19] J.L. Lyon, K.J. Stevenson, Electrochim. Acta 53 (2008) 6714.
[20] P.J. Britto, K.S.V. Santhanam, P.M. Ajayan, Bioelectrochem. Bioenerg. 41 (1996) 121.
[21] A. Abbaspour, A. Ghaffarinejad, Electrochim. Acta 53 (2008) 6643.
[22] T. Garcia, E. Casero, E. Lorenzo, F. Pariente, Sens. Actuators B 106 (2005) 803.
[23] D.L. Lu, A. Cagan, R.A.A. Munoz, T. Tangkuaram, J. Wang, Analyst 131 (2006) 1279.
[24] Z. Gao, G. Wang, P. Li, Z. Zhao, Electrochim. Acta 36 (1991) 147.
[25] J. Joseph, H. Gomathi, G.P. Rao, J. Electroanal. Chem. 304 (1991) 263.
[26] R.E. Sabzi, A. Hasanzadeh, K. Ghasemlu, P. Heravi, J. Serb. Chem. Soc. 72 (2007) 993.
[27] S. Dong, Z. Jin, Electrochim. Acta 34 (1989) 963.
[28] A. Eftekhari, Anal. Lett. 34 (2001) 541.
[29] J. Joseph, H. Gomathi, G.P. Rao, J. Electroanal. Chem. 431 (1997) 231.
[30] M. Jiang, X. Zhou, Z. Zhao, J. Electroanal. Chem. 287 (1990) 389.
[31] M.S. Lin, W.C. Shih, Anal. Chim. Acta 381 (1999) 183.
[32] A.P. Baioni, M. Vidotti, P.A. Fiorito, S.I. Córdoba de Torresi, J. Electroanal. Chem. 622 (2008) 219.
[33] S.-M. Chen, C.-M. Chan, J. Electroanal. Chem. 543 (2003) 161.
[34] F. Wang, J. Wang, H. Chen, S. Dong, J. Electroanal. Chem. 600 (2007) 265.
[35] D. Ravi Shankaran, S. Sriman Narayanan, Sens. Actuators B 55 (1999) 191.
[36] C. Chinvongamorn, K. Pinwattana, N. Praphairaksit, T. Imato, O. Chailapakul, Sensors 8 (2008) 1846.
[37] R. Carballo, V.C. Dall'Orto, A. Lo Balbo, I. Rezzano, Sens. Actuators B 88 (2003) 155.
[38] E. Mataix, M.D. Luque de Castro, Analyst 123 (1998) 1547.
[39] E. Mataix, M.D. Luque de Castro, Fresenius J. Anal. Chem. 365 (1999) 377.

[40] H. Sulistyarti, T.J. Cardwell, M.D. Luque de Castro, S.D. Kolev, Anal. Chim. Acta 390 (1999) 133.
[41] I. Papaefstathiou, M.D. Luque de Castro, Anal. Lett. 28 (1995) 2063.
[42] Association of Official Analytical Chemists, Official Methods of Analysis of the Association of Official Analytical Chemists, 15th Edn., AOAC, Inc, Arlington, 1990, 1298p.
[43] R.T. Kachoosangi, M.M. Musameh, I. Abu-Yousef, J.M. Yousef, S.M. Kanan, L. Xiao, S.G. Davies, A. Russell, R.G. Compton, Anal. Chem. 81 (2009) 435.
[44] A.S. Arribas, E. Bermejo, M. Chicharro, A. Zapardiel, G.L. Luque, N.F. Ferreyra, G.A. Rivas, Anal. Chim. Acta 577 (2006) 183.
[45] D.C. Harris (Ed.), Quantitative Chemical Analysis, 5th Edn., W. H. Freeman and Company, New York, 2000, 899p.