Talanta 82 (2010) 2003–2006

Contents lists available at ScienceDirect

Talanta

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

Short communication

Rapid flow injection method for the determination of sulfite in wine using the permanganate–luminol luminescence system

Mercedes Villar Navarrro, María Ramos Payán, Miguel Angel Bello López*, Rut Fernández-Torres, Manuel Callejón Mochón

Department of Analytical Chemistry, Faculty of Chemistry, University of Seville, c/Prof. García González s/n, 4012 Seville, Spain

ARTICLE INFO

Article history:
Received 14 June 2010
Received in revised form 29 July 2010
Accepted 17 August 2010
Available online 22 August 2010

Keywords:
Sulfite
Chemiluminescence
Permanganate–luminol
Flow-injection analysis
Wine

ABSTRACT

A simple, rapid and sensitive chemiluminescence method for the determination of sulfite has been developed by combining flow-injection analysis and its sensitizing effect on the known chemiluminescence emission produced by the oxidation of luminol in alkaline medium; in this work permanganate has been proposed as oxidizing reactive. The optimum conditions for the chemiluminescence emission were established. The chemiluminescence was proportional to the sulfite concentration over the range 1.6×10^{-5} and 4.0×10^{-4} mol L^{-1}. The detection limit was 4.7×10^{-6} mol L^{-1} of sulfite. The method has been satisfactorily used for the determination of free and bound sulfite in wines.

1. Introduction

Sulfites are frequently used as preservatives in several foods and beverages, and traditionally they are essentials in the winemaking as anti-oxidants and bacterial control agents. Even in the old Greek and Roman costumes of winemaking sulfites were commonly used. Free sulfur dioxide is the active preservative, but sulfur dioxide bound to organic compounds in the wine can replenish the free form; this shift over time influences product flavour and acceptability. The determination of free and total sulfites is common in wine laboratories because these parameters affect the sensory properties and evolution of wines. A negative aspect of the use of sulfites as food preservatives is that they can cause allergenic responses in asthmatics and skin sensitivity [1]. Also, they interact with some vitamins such as thiamin, pyridoxal, nicotinamide, and folic acid [2].

There are several methods to determine sulfites which can be used to their determination in wine. The traditional method applied to foods and beverages is the Monier–Williams method modified by Rankine and Pocock [3–6] which involves an acid distillation step to release sulfite as sulfur dioxide, which is transferred, using a carrier gas stream, to an oxidizing trapping containing hydrogen peroxide in an alkaline medium; then the sulfite content is determined as sulfate using any adequate acid-base titration or gravimetric procedure. The p-rosanilie method [7] has been accepted by the AOAC for its application in foods but the toxicity of the reagents used could be an important limitation. Sulfites have been also determined by ion chromatography using several detection systems [8,9], capillary electrophoresis [10,11], linear sweep voltammetry [12], on-line microdistillation with conductimetric detection [13] or stopped-flow system measuring the light scattering intensity versus time [14]; most of them have been applied to the sulfite determination in wine.

Chemiluminescence (CL) analysis has received attention in different fields due to its high sensitivity, wide linear range, and simple instrumentation. When it is coupled with flow-injection analysis (FIA) the CL-based FIA method is a cheap, rapid, simple, and reproducible detection procedure and, therefore, has been successfully applied to the detection of many compounds in a great variety of matrices.

They are some sulfite CL determinations applied to a variety of matrices that use diverse luminescence systems: acidic permanganate/riboflavin [15,16] or 3-cyclohexylaminopropanesulfonic acid [15], tris(2,2%-bipyridyl)ruthenium(II)-permanganate [17], platinum(II) complex of coproporphyrin-I and bovine serum albumin (PtCP–BSA) [18], and several that use the known CL properties of luminol: inhibition of electroluminescence of luminol [19], electrostatically immobilized luminol on an anion exchange column [20], sulfite induced autoxidation of Ni(II)/tetraglycine complex in the presence of luminol [21], even an iodometric determination of

* Corresponding author. Tel.: +34 954557172; fax: +34 954557168.
E-mail address: mabello@us.es (M.A.B. López).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.08.021

sulfite in an alkaline medium using luminol as chemiluminescence indicator [22].

Some references concerning to both free and total assessment of sulfite in wines using continuous flow techniques using several detection principles were published but only three of them use any luminescence system: oxidation of the disulfite-mercurate complex with cerium(IV) in an acidic solution in the presence of riboflavin sulfate [23], suppression of CL from H_2O_2/luminol/EDTA/horseradish peroxidase [24] and CL from the sulfite–Na_2CO_3–$NaHCO_3$–Cu^{2+} system [25].

The strong CL emission by the oxidation of luminol in alkaline medium is one of the best known and most efficient CL reactions and the CL mechanism has been previously described [26] that can be summarized in OH^- removes the nitrogen protons leaving a negative charge which moves onto the carbonyl oxygen to form an enolate, then the action of the oxidant leads to activated 3-aminophthalate (3-APA*) which emits a strong luminescence. Different oxidizing can be used, such as hydrogen peroxide, molecular oxygen, hypochlorite or permanganate, mainly in the presence of some type of initiator or catalyst such as peroxidase, hexacyanoferrate(III), and compounds or metal ions. In this work, a new FIA–CL method using permanganate as oxidizing has been developed for the determination of sulfite which has been applied to the free and combined sulfite determination in wine.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical-reagent grade or better. All solutions and dilutions were prepared with ultrapure water (Milli-Q, Millipore, Bedford, MA). Luminol was purchased from Sigma (Sigma–Aldrich, Madrid, Spain) and the rest of products were obtained from Merck (Darmstadt, Germany).

Working solution of 1.2×10^{-4} M $KMn0_4$ was prepared daily by diluting a 3×10^{-3} M $KMn0_4$ stock solution. Solution of 2×10^{-5} M luminol in 4×10^{-3} M NaOH was prepared daily and kept in the darkness. Working solutions of sulfite were prepared by adequate dilutions from a 500 μg mL^{-1} Na_2S0_3 stock solution prepared daily.

2.2. Apparatus

The FIA system used in this work is a very simple configuration consisting on one way for the $KMnO_4$ solution and another for the luminol solution when sulfite solutions were injected. To deliver flow streams, a peristaltic pump Minipuls 3 from Gilson (Gilson Inc., Middleton, WI, USA) was used. Polytetrafluoroethylene (PTFE) tubing (0.8 mm i.d.) was used to connect all components in the flow system. A 100 μL loop was placed in the injection valve. The CL signal was measured by a ChemLab Chemiluminescence Detector model CL2 (Camspec, Cambridge, UK) where the carrier streams were mixed through a Y-shaped element previously to the 60 μL/5 mm pathlength glass flow cell. CL data were acquired with a personal computer using Clarity Lite software (DataApex Ltd., Prague, The Czech Republic).

2.3. Proposed procedure

By keeping the six-way valve in washing position, permanganate and luminol solutions were continuously pumped into the manifold at a flow rate of 1.2 mL min^{-1}. 100 μL sulfite solution (containing 1.6×10^{-5} to 4.0×10^{-4} mol L^{-1}) was injected into the luminol stream from the valve loop. The content of sulfite was determined from the calibration plot of CL emission intensity versus sulfite concentration.

2.4. Analysis of wine samples

In order to obtain the free and bound sulfite content, wine samples were submitted to the Monier–Williams distillation procedure lightly modified. A stream of nitrogen was pumped at room temperature into a 100 mL aqueous solution containing 6 mL of wine and 10 mL of 85% H_3PO_4; free SO_2 was collected on 2×10^{-3} M NaOH. The remaining acid aqueous solution was boiled to collect the bound SO_2 on 2×10^{-3} M NaOH. Free and bound SO_2 solutions were directly injected into the FIA system for their determination. To evaluate the proposed procedure, the results obtained were compared with those from the Ripper–Jaulmes procedure [27,28].

3. Results and discussion

As has been indicated in Section 1, the reaction between luminol and several oxidants in alkaline media yields, in general, strong luminescence signals. Also has been previously described by several authors that the presence of foreign substances can increase the emission which can be used to their determination [29–33]. In our case, we have checked that the presence of sulfite enhances the CL signal.

3.1. Optimization of the experimental conditions

Several tests were performed to choose the best experimental conditions in order to obtain maximum CL signals. The effect of NaOH concentration on CL intensity was tested from 4×10^{-4} to 6×10^{-3} M, using 10^{-5} M luminol and 10^{-4} M $KMnO_4$ at a flow rate of 1.0 mL min^{-1}. Fig. 1 shows the effect of the NaOH concentration when 1.5×10^{-4} M sulfite was injected. As can be seen, the CL signal strongly increases below approximately 3.5×10^{-3} M and can be considered practically constant up to this concentration. A NaOH concentration of 4×10^{-3} M was chosen as optimum and fixed for the next optimization tests.

The effect of luminol concentration was investigated over the range of 4×10^{-6} to 4×10^{-5} M. The baseline increased with increasing luminol concentration. Fig. 1 shows the CL obtained when 1.5×10^{-4} M sulfite was injected into a stream of variable concentration of luminol in 4×10^{-3} M NaOH and was mixed with 10^{-4} M $KMnO_4$ at a flow rate of 1.0 mL min^{-1}. A strong CL increasing can be observed for luminol concentrations lower than 10^{-5} M; from this concentration a lower CL increasing was measured. An optimal concentration of luminol of 2×10^{-5} M was chosen for subsequent experiments due that higher luminol concentration allows high baseline signals.

By fixing the NaOH and luminol concentrations to the previously optimized ones, $KMnO_4$ concentration was increased and CL signal measured when 1.5×10^{-4} M sulfite was injected (Fig. 1). As can be seen, the CL increased with the permanganate concentration reaching a maximum value from 5×10^{-5} to 1.7×10^{-4} M; higher permanganate concentrations resulted in a decrease of the emission intensity, which could be due to a permanganate self-absorption [34,35] or formation of a precipitate from $KMnO_4$ in basic medium [26]. A $KMnO_4$ concentration of 1.2×10^{-4} M was selected as optimum.

The flow rate is an important factor in flow-injection CL systems; an optimum flow rate is necessary for maximum collection of the emitted light in the flow cell to deliver the excited product. The effect of the flow rate on CL emission was tested when 1.5×10^{-4} M sulfite was injected in the FIA system using the previously optimized conditions. As can be observed in Fig. 1, the highest CL intensity was achieved when the flow rate was between 0.9 and 1.4 mL min^{-1} allowing well defined signals. A flow rate of 1.2 mL min^{-1} was chosen for this work.

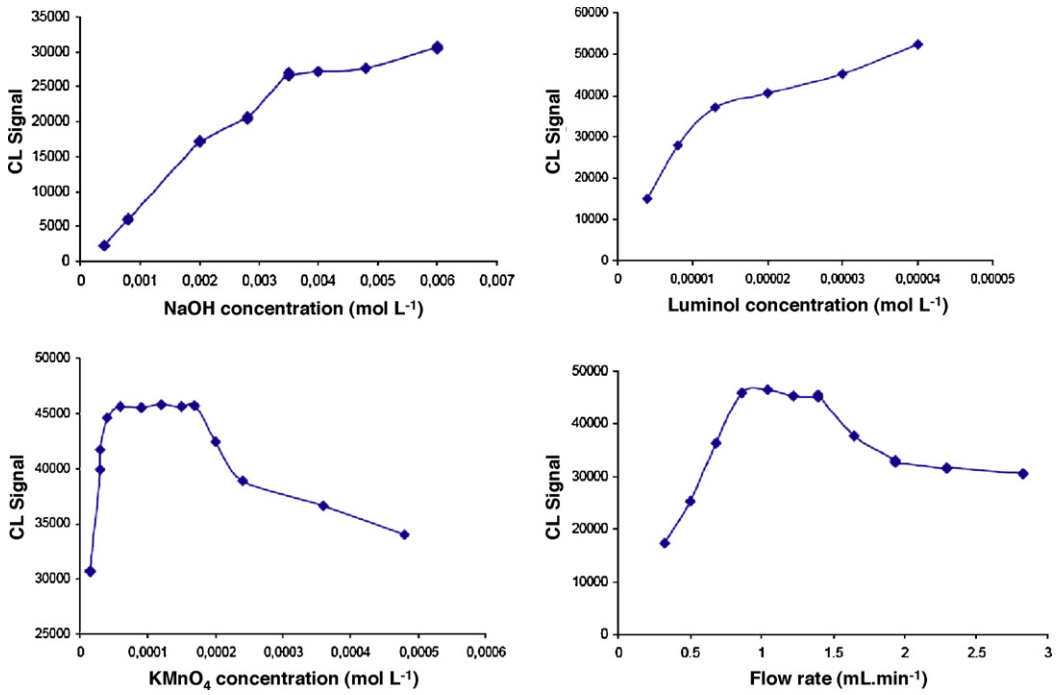

Fig. 1. Influence of the analytical parameters on the CL signal.

Table 1
Analysis of wine samples.

Sample	Free SO_2				Bound SO_2			
	Proposed method [a]	Ripper–Jaulmes method[a]	t_{exp}	F_{exp}	Proposed method[a]	Ripper–Jaulmes method[a]	t_{exp}	F_{exp}
White wine 1	28 ± 4	23 ± 1	2.10	16.0	85 ± 3	80 ± 2	2.40	2.25
White wine 2	10 ± 3	11 ± 2	0.48	2.25	44 ± 3	45 ± 2	0.48	2.25
Red wine 1	23 ± 3	21 ± 2	0.48	2.25	96 ± 3	98 ± 4	1.09	9.00
Red wine 2	31 ± 2	33 ± 2	0.61	1.00	68 ± 5	65 ± 3	0.96	12.5
			2.78[b]	39[b]			2.78[b]	39[b]

[a] Average of three determinations ± standard deviation (mg L^{-1} SO_2).
[b] Critical values for F and t (P = 0.05).

3.2. Linearity, sensitivity and precision for the proposed procedure

In order to obtain the response linearity, a 10-point (in triplicate) calibration curve, based on peak areas, was constructed using a least-square linear regression analysis of aqueous solutions of sulfite at concentrations ranging between 3.0×10^{-5} and 4.0×10^{-4} M. A linear relationship was obtained with correlation coefficients $r \geq 0.999$. In order to test the quality of straight lines and to achieve the true linear range, the procedure of Huber [36] was applied. The concentration range studied was linear and the corresponding calibration equation was $y = (3.07 \times 10^8 \pm 0.05 \times 10^8)x + (2033 \pm 483)$.

Limits of detection and quantitation were calculated as the minimum concentration of analyte giving peak whose signal-to-noise ratio is 3 and 10, respectively. LOD was 4.7×10^{-6} M and LOQ was 1.6×10^{-5} M (R.S.D. 4.2% calculated from a triplicate).

To evaluate the repeatability and the intermediate precision, aqueous sulfite samples (n = 3) at three concentration levels 8.0×10^{-5}, 1.5×10^{-4} and 2.3×10^{-4} M were measured in one single day and 2 day per week during 1 month, respectively. The repeatability, expressed as relative standard deviation, was in the range 2.8–3.5%. Intermediate precision, also expressed as relative standard deviation, was in the range 3.2–4.6%.

3.3. Analysis of wine samples

Four wine samples (two white and two red ones) were analyzed applying the proposed analytical procedure after the extraction procedure for free and bound sulfite described in Section 2. Samples were analyzed in triplicate and the results are shown in Table 1. The samples were also analyzed by the Ripper–Jaulmes method [28]. As can be seen (Table 1), good agreement was found between the volumetric and the proposed method, statistically tested using two significance tests, t-test for the comparison of two experimental means and the F-test for the comparison of standard deviations [37].

4. Conclusions

This study presents a rapid chemiluminescence determination of sulfite using its sensitizing effect on the alkaline luminol/permanganate reaction into a FIA configuration. The optimization of the experimental parameters has been carried out. The applicability of the method for the free and bound sulfite determination in wine has been demonstrated. The simplicity of the analysis procedure makes the developed method as an attractive alternative to other ones.

References

[1] A.A. Fisher, Cutis 44 (1989) 283–284.
[2] L. Pizzoferrato, G. Di Lullo, E. Quattrucci, Food Chem. 63 (1998) 275–279.
[3] AOAC Official Method 990.28, AOAC Official Methods of Analysis, AOAC, Arlington, VA (2000) Chapter 47, pp. 29–30.
[4] G. Monier-Williams, Rep. Publ. Health Med. Subj. 43 (1927) 1.
[5] G. Monier-Willians, Br. Food J. 29 (1927) 51–53.
[6] B.C. Rankine, K.F. Pocock, Brewing Spirit Rev. (1970) 40.
[7] AOAC Official Method 963.20, AOAC Official Methods of Analysis, AOAC Arlington, VA (2000) Chapter 47, pp. 26–27.
[8] Y. Miura, M. Hatakeyama, T. Hocino, P.R. Haddad, J. Chromatogr. A 956 (2002) 77–84.
[9] S. Fäldt, B. Kalberg, W. Frenzel, Fresen. J. Anal. Chem. 371 (2001) 425–430.
[10] L. Saavedra, C. Barbas, Electrophoresis 24 (2003) 2235–2243.
[11] M. Masár, M. Danková, E. Ölvecká, A. Stachurová, D. Kaniansky, B. Stanilawski, J. Chromatogr. A 1026 (2004) 31–39.
[12] M. Scampicchio, N.S. Lawrence, A. Arecchi, S. Mannino, Electroanalysis 20 (4) (2008) 444–447.
[13] S. McLeod, D.E. Davey, Anal. Chim. Acta 600 (2007) 72–77.
[14] R. Rodríguez-Diaz, M. Aguilar-Caballos, A. Gómez-Hens, Am. J. Agric. Food Chem. 52 (2004) 7777–7781.
[15] S.A. Al-Tamrah, A. Townshend, A.R. Wheatley, Analyst 112 (6) (1987) 883–886.
[16] W. Wei Qina, Z. Zhangb, C. Zhang, Anal. Chim. Acta 361 (3) (1998) 201–203.
[17] M. Hui, W. Fengwu, H. Zhike, Z. Yun'e, Talanta 48 (1999) 571–577.
[18] D. Papkovskya, M.A. Uskovaa, G.V. Ponomarevb, T. Korpelac, S. Kulmalac, G.G. Guilbaulta, Anal. Chim. Acta 374 (1) (1998) 1–9.
[19] J. An, J. Lin, X. Xu, Fenxi Shiyanshi 12 (5) (1993) 4–7.
[20] Q. Wei, Z. Zhu-Jun, Z. Cheng-Jie, Fresen. J. Anal. Chem. 361 (1998) 824–826.
[21] R.L. Bonifácio, N. Coichev, Anal. Chim. Acta 517 (2004) 125–130.
[22] N.M. Lukovskaya, L.V. Markova, Z. Anal. Khim. 24 (12) (1969) 1893–1894.
[23] J.L. Burguera, M. Burguera, Anal. Chim. Acta 214 (1988) 429–432.
[24] Y.L. Huang, J.M. Kim, R.D. Schmid, Anal. Chim. Acta 266 (2) (1992) 317–323.
[25] L. Jin-Ming, H. Toshiyuki, Anal. Chim. Acta 323 (1996) 69–74.
[26] W. Zhouping, Z. Zhujun, F. Zhifeng, Z. Xiao, Anal. Bioanal. Chem. 378 (2004) 834–840.
[27] M. Ripper, J. Prakt. Chem. (Leipzig) 46 (2) (1893) 428–473.
[28] P. Jaulmes, G. Hamelle, Ann. Falsif. Expert. Chim. 54 (1961) 338–346.
[29] Z. Wang, Z. Zhang, Z. Fu, X. Zhang, Anal. Bioanal. Chem. 378 (2004) 834–840.
[30] J.F. Huertas-Pérez, A.M. García-Campaña, A. González-Casado, L. Gámiz-Gracia, Luminescence 19 (2004) 222–224.
[31] J. Du, J. Lu, Luminescence 19 (2004) 328–332.
[32] S. Hu, W. Liu, Y. Huang, W. Shu, J. Cao, Luminescence 21 (2006) 245–250.
[33] Y. Li, J. Lu, Luminescence 21 (2006) 251–255.
[34] J.L. López-Paz, A. Townshend, Anal. Commun. 33 (1996) 31–33.
[35] A. Campiglio, Analyst 123 (1998) 1053–1056.
[36] L. Huber, Validation and Quantification in Analytical Laboratories, Interpharm Press, East Englewood Co., USA, 1998.
[37] J.N. Miller, J.C. Miller, Statistics and Chemometrics for Analytical Chemistry, 4th edition, Prentice Hall-Pearson Education Limited, Essex, England, 2000.