Speciation of free and total sulphite in wines by capillary electrophoresis

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A capillary electrophoretic (CE) technique was developed for the selective determination of free and total sulphite in beverages. The proposed method is based on the pre-column derivatization of sulphite with iron(III)-1,10-phenanthroline complex ($Fe(phen)_3^{3+}$) and CE determination of the $Fe(phen)_3^{2+}$ formed. The optimal conditions for the separation and derivatization reaction were established by varying electrolyte composition, pH, reagent concentration and reaction time. The optimized separations were performed in 20 mmol/l imidazole-acetate electrolyte (pH 5.5) using direct UV detection at 254 nm. Valid calibration is demonstrated in the range 8×10^{-6}–1×10^{-3} mol/l of sulphite. The detection limit was 1×10^{-6} mol/l. The proposed method was applied to the free and bound sulphite in wines. The recovery tests established for several samples were within the range 100 ± 8%.

Key words: capillary electrophoresis, speciation, sulphite, wines

INTRODUCTION

Sulphur dioxide is added to a range of beverages to prevent oxidation and to prolong the shelf life of products. It is usually present in beverages either free, as HSO_3^- and SO_2, or bound to carbonyl or unsaturated compounds and/or phenol derivatives [1]. The sum of free and bound sulphite is designated as total sulphite. Determination of free and total sulphite is routinely performed in beverages, since its maximum value is established by legislation in many countries.

Many analytical techniques are used to determine free and/or total sulphite in foods. Redox processes are generally involved in these determinations. The titration of sulphite with iodine best represents the classical approach to sulphite determination [2, 3]. Free sulphite is determined by direct titration without sample pre-treatment, whereas for determination of total sulphite acid or alkali distillation it is used prior the titration. The simplicity of this method has significant limitations in terms of sensitivity and selectivity when dealing with authentic, real samples. More sensitive spectrophotometric methods involve redox reactions with sulphite in which a coloured compound such as fuchsin [4, 5] or Fe(II) complex with 1,10-phenantroline [6, 7] is formed or decomposed. However, the spectrophotometric method would not be suitable for the analysis of highly coloured samples such as red wines and certain fruit juices.

Separation techniques such as ion chromatography (IC) and capillary electrophoresis (CE) can offer significant advantages when considering coloured samples and samples containing other redox active species. IC and CE methods for determination of sulphur species including sulphite have been reviewed recently [8]. A great disadvantage of IC with conductivity detection or CE with indirect UV detection commonly used for determination of sulphite in real samples is an interference by the high concentrations of co-eluting matrix anions like sulphate. In addition, accurate determination of sulphite is problematic due to the ease with which it is oxidized by oxygen during the separation [9, 10]. Consequently, rapid pre-analysis conversion of sulphite ions to a stable derivative should prevent degradation of this analyte during analysis. Moreover, such derivatization procedure in conjunction with an appropriate separation technique could significantly improve the selectivity and detectability.

In this paper, we report on the capillary electrophoretic method for a sensitive and selective speciation of free and bound sulphite based on the pre-capillary derivatization with iron(III)-1,10-phenanthroline complex, CE separation and direct UV detection of the $Fe(phen)_3^{2+}$ formed.

EXPERIMENTAL

Separations were performed on a P/ACE 2100 apparatus (Beckman Instruments Inc., Fullerton, CA, USA) equipped with a UV detector with wavelength filters (200, 214, 230 and 254 nm). Fused silica capillary (Polymicro Technology, Phoenix, AZ, USA) of 75 μm i.d. (375 μm o.d.) and 57 cm total length (50 cm to the detector) was used. Samples were injected in the hydrodynamic mode by overpressure (3.43×10^3 Pa). System Gold software was used for data acquisition. UV detection was employed at 254 nm. All experiments were conducted at 25 °C.

All electrolyte and standard solutions were prepared using doubly distilled helium degassed water. Stock sulphite solutions (about 0.01 mol/l) were prepared daily by dissolving 0.126 g of Na_2SO_3 in 100 ml of oxygen-free water, and were standardized by iodometric titration before use. Stock solution (0.01 mol/l) of Fe(III) was prepared by dissolving $FeNH_4(SO_4)_2 \cdot 12H_2O$ in 0.01 mol/l of sulphuric acid. Stock solution (0.01 mol/l) of 1,10-phenanthroline (*phen*) was prepared by dissolving *phen* in water and acidifying (pH~5.0) with 0.01 mol/l of sulphuric acid. Stock solution (0.01 mol/l) of Fe(II) was prepared daily by dissolving $FeSO_4 \cdot 7H_2O$ in 0.01 mol/l of sulphuric acid. Standard solutions of Fe(*phen*)$_3^{2+}$ chelate used for the optimization of CE conditions were prepared by dilution of appropriate volumes of Fe(II) and *phen* stock solutions.

All electrolyte solutions were filtered through a 0.2 μm membrane filter. The capillary was rinsed with 1.0 mol/l sodium hydroxide and water for 5 min, then equilibrated with carrier electrolyte for 10 min at the beginning of each day. Between all electrophoretic separations the capillary was rinsed for 2 min with carrier electrolyte.

RESULTS AND DISCUSSION

Sulphite stability

As already mentioned, accurate determination of sulphite in aqueous solution has been problematic due to the ease with which it is oxidized by dissolved oxygen to sulphate. The time in which sulphite is reported to be stable varies widely from 15 min [11, 12] to 2 weeks [13]. In order to evaluate the stability of a sulphite, standard sulphite solutions were prepared in water degassed by different procedures. The changes of the amount of sulphite with respect to time were examined by iodometric titration. The results obtained are compared in Table 1. As can be observed, a decrease of 17% in the sulphite amount 2 h after solution preparation and of 67% after 24 h shows a rapid conversion of SO_3^{2-} into SO_4^{2-} in an aqueous solution prepared in doubly distilled water without degassing. After 36 h, almost all sulphite present in the solution is oxidized to sulphate. In the solution additionally degassed by ultrasonication, oxidation of sulphite was significantly slower: during 24 h after preparation the amount of sulphite decreased by about 21%. A very similar sulphite oxidation rate was also observed in the vacuum degassed solution. The most effective degassing method was helium sparging: this technique provided a good stabilizing effect for up to 24 h. These results demonstrate that in order to prevent sulphite oxidation prior and during analysis all the procedures should be performed as soon as possible after sampling using thoroughly degassed solutions. Another way to overcome stability problems is rapid and complete conversion of sulphite into a stable derivative.

Table 1. **Stability of sulphite standard prepared in water degassed by different procedures (sulphite concentration 0.5 mmol/l)**

Time (h)	Sulphite signal recovery (%)			
	Without degassing	Vacuum degassing	Ultrasonic degassing	Helium degassing
0.5	94	97	98	99
1	91	95	96	99
2	83	94	95	97
5	68	93	90	98
10	52	89	87	98
24	33	82	79	96
36	12	75	72	87

Optimization of CE conditions

For maximum efficiency and detection sensitivity, the mobility of the electrolyte co-ion must be as close as possible to the mobility of the analyte [14]. In addition, buffering of the electrolyte is essential for reproducible and rugged separations. The effective mobility of Fe(*phen*)$_3^{2+}$ chelate measured at pH 5.0 was found to be 2.48×10^{-4} cm^2/V s. Consequently, it would be beneficial to use an electrolyte co-ion with a moderate mobility. In this work, we tested two electrolyte co-ions: ethylenediamine and imidazole. Experiments were performed in electrolyte containing 20 mmol/l of CH_3COOH neutralized with appropriate base to pH 5.0. Significantly sharper Fe(*phen*)$_3^{2+}$ peak shapes using imidazole co-ion were obtained. Based on these results, imidazole was used in all further investigations. Unfortunately, preliminary results indicate that the difference in effective mobilities of Fe(*phen*)$_3^{2+}$ and free *phen* is negligible and under the conditions used both cations partially comigrate. Consequently, accurate quantification of

the Fe(*phen*)$_3^{2+}$ chelate is impossible under these conditions. Increasing the pH of the electrolyte provides additional possibilities to improve the separation selectivity. Figure 1 shows the influence of electrolyte pH on the migration times of the cations studied. At pH above 5, the *phen* cation becomes less protonated (pK_a = 4.9) and its mobility decreases, whereas the mobility of iron(II)–*phen* chelate does not change significantly. The best overall separation was obtained in the pH range 5.5–6.0. Under these conditions, free *phen* migrates much slower than the analyte studied. Figure 2 shows the separation of both cations in less than 5 min under optimum conditions. It should be noted that the less stable Fe(*phen*)$_3^{3+}$ chelate completely decomposes during the separation process and thus cannot be detected.

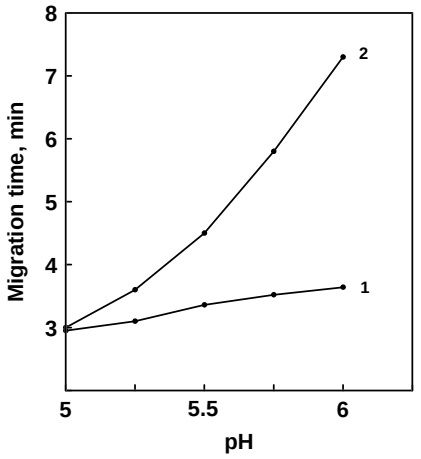

Fig. 1. Effect of electrolyte pH on the migration time of Fe(*phen*)$_3^{2+}$ (1) and free *phen* (2). Electrolyte, 20 mmol/l CH_3COOH neutralized with imidazole. Applied voltage, 30 kV; direct UV detection at 254 nm

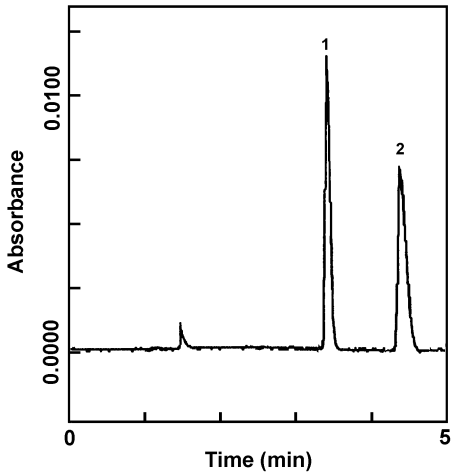

Fig. 2. Electropherogram of a standard Fe(*phen*)$_3^{2+}$ and *phen* solution under optimum conditions. Peaks: 1 – Fe(*phen*)$_3^{2+}$; 2 – *phen*. Electrolyte, 20 mmol/l CH_3COOH neutralized with imidazole to pH 5.5

Derivatization

The proposed derivatization procedure requires quantitative or at least reproducible reaction between sulphite and Fe(*phen*)$_3^{3+}$ chelate. To establish the optimal reaction conditions, the influence of pH, concentration of Fe(*phen*)$_3^{3+}$, and time on the derivatization reaction was investigated. The effect of pH on the peak area of Fe(*phen*)$_3^{2+}$ formed after derivatization of sulphite standards at three concentration levels is demonstrated in Fig. 3. The concentration of Fe(*phen*)$_3^{3+}$ was kept constant at 0.5 mmol/l. The peak areas were measured at 20 min after the addition of sulphite standard. No significant changes in peak area occur in the pH range 4.0–6.0. At pH lower than 4.0 the peak area of iron(II) chelate decreases, probably due to partial volatilization of SO_2.

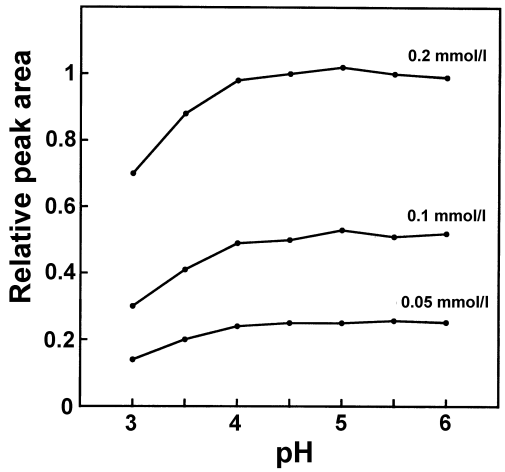

Fig. 3. Effect of pH on the peak area of the Fe(*phen*)$_3^{2+}$ chelate formed for three sulphite concentrations

To establish the optimal amount of the reagent required for the complete derivatization of sulphite, the effect of Fe(*phen*)$_3^{3+}$ concentration on the derivatization reaction was investigated. In this experiment the Fe(*phen*)$_3^{3+}$ concentration was increased, whereas the sulphite concentration was maintained constant. The results obtained for three different sulphite concentrations showed that the peak area of the Fe(*phen*)$_3^{2+}$ formed increased continuously with increasing the concentration of Fe(*phen*)$_3^{3+}$, and reached a plateau for Fe(*phen*)$_3^{3+}$ and sulphite concentration ratios higher than 2–2.5. It was observed that rather high concentrations of the reagent (up to 10 mmol/l) showed no significant effect on the reaction studied.

Finally, the effect of reaction time on the signal response was examined for three sulphite concen-

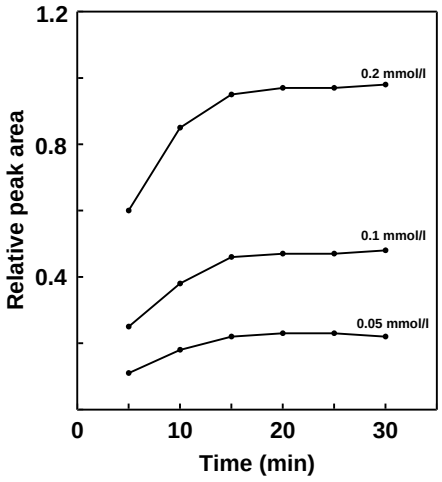

Fig. 4. Effect of reaction time on the peak area of the Fe(*phen*)$_3^{2+}$ chelate formed for three sulphite concentrations

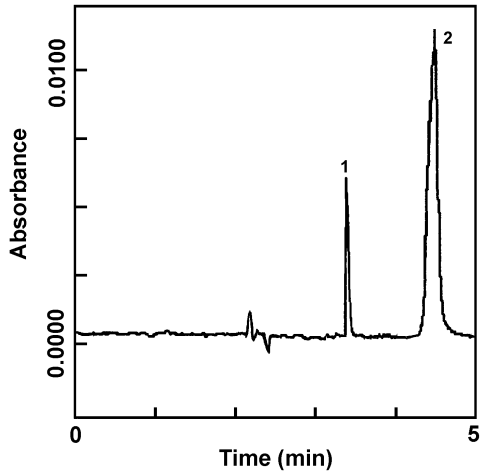

Fig. 5. Electropherogram of 1:10 diluted sparkling apple wine (cider) sample. Conditions as in Fig. 2. Peaks: 1 – Fe(*phen*)$_3^{2+}$; 2 – *phen*

trations (Fig. 4). As can be seen, under the conditions used the derivatization reaction is complete within 15 min. No degradation of the derivative was observed up to 24 hours, the maximum reaction time studied. A reaction time of 20 min was used in our work.

Sample analysis

Several analytical performance characteristics important for quantitative analysis were measured. To determine the migration time and peak area repeatability, solutions containing 0.1 mmol/l of sulphite were derivatized and then analyzed sequentially six times. The relative standard deviation (RSD) of the migration times was 0.27%, while the RSD of the peak areas was 2.5%. The linearity of the calibration curve was measured by triplicate injections of derivatized sulphite standards. As a criterion of linearity, deviation within 5% of the mean response factor was used. Valid calibration (r^2 = 0.998) is demonstrated in the range $8 \times 10^{-6} - 1 \times 10^{-3}$ mol/l of sulphite. The detection limit determined for 20 s hydrodynamic injection was 1×10^{-6} mol/l (three times the baseline noise).

To evaluate the proposed CE system for the real samples, it was applied to the determination of free and total sulphite in wines. For determination of free sulphite, into a 25.0 ml volumetric flask 2.5 ml of 0.01 mol/l Fe(III) solution was pipetted followed by addition of 7.5 ml of 0.01 mol/l *phen* solution and 2.5 ml of 0.025 mol/l acetate buffer (pH 5.0). Then exactly 2.50 ml of wine sample was added. After 20 min of equilibration, the sample solution was diluted (the final dilution was 10-fold) and analysed. For the determination of total sulphite, the same procedure was applied with a prior alkaline hydrolysis to release the bound sulphite [15]. The standards were treated in exactly the same way as the wine aliquots. In order to prevent errors caused by the reduction of the Fe(*phen*)$_3^{3+}$ with other reductors often present in wine samples, all the determinations were corrected by the blank in which sulphite was masked with formaldehyde according to the procedure described in [16]. Figure 5 shows the electropherogram obtained for a sparkling apple wine sample. Several wine samples were analyzed by the proposed CE method using a standard addition procedure and the results are presented in Table 2. As can be observed, the recoveries in all the cases were within the range 100 ± 8%. The proposed method appears to be a good alternative to iodometric and colorimetric procedures for determining sulphite in highly coloured samples such as red wines, certain fruit juices, etc.

Table 2. **Determination of free and total sulphite in wines (n = 3)**

Sample	Sulphite	Found, mg/l	Added, mg/l	Found total, mg/l	Recovery, %
White wine	Free	25.8	10.0	35.2	94
	Total	123	50.0	169	92
Red wine	Free	15.6	10.0	26.0	104
	Total	135	50.0	183	96
Cider	Free	48.2	10.0	57.9	97
	Total	169	50.0	220	102

CONCLUSIONS

A new capillary electrophoretic technique has been developed for the selective determination of free

and total sulphite in beverages. The pre-capillary derivatization of sulphite prevents degradation of this analyte during analysis. Furthermore, such derivatization procedure in conjunction with the separation technique significantly improves the selectivity and detectability. In comparison with the standard titrimetric and spectrophotometric techniques, this method can be applied to the analysis of highly coloured samples such as red wines and certain fruit juices.

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LAISVO IR BENDRO SULFITO VYNUOSE NUSTATYMAS KAPILIARINĖS ELEKTROFOREZĖS METODU

S a n t r a u k a

Sulfitui nustatyti pasiūlytas ir optimizuotos kapiliarinės elektroforezės (KE) metodas. Nustatymas pagrįstas sulfito jonų oksidacija Fe(III)-1,10-fenantrolino kompleksu ir susidariusio Fe(II)-1,10-fenantrolino komplekso nustatymu KE metodu. Optimizuotos geležies (II) komplekso nustatymo sąlygos: 20 mmol/l imidazolo-acetato elektrolitas (pH 5,5), tiesioginis detektavimas esant 254 nm bangos ilgiui. Ištirta pH, reagento koncentracijos ir reakcijos trukmės įtaka derivatizacijos reakcijai. Išmatuotos pagrindinės analizinės charakteristikos: kalibracinė kreivė yra tiesinė sulfito koncentracijų intervale $8 \cdot 10^{-5}$–$1 \cdot 10^{-3}$ mol/l; aptikimo riba siekia $1 \cdot 10^{-6}$ mol/l. Metodas pritaikytas laisvam ir bendram sulfitui vynuose nustatyti.