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Capillary electrophoretic determination of sulfite using the zone-passing technique of in-capillary derivatization

Giedre Jankovskiene, Zydrunas Daunoravicius, Audrius Padarauskas*

Department of Analytical and Environmental Chemistry, Vilnius University, Naugarduko 24, LT-2006 Vilnius, Lithuania

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Abstract

A new capillary electrophoretic (CE) method was developed for the simple and selective determination of sulfite. The proposed method is based on the in-capillary derivatization of sulfite with iodine using the zone-passing technique and direct UV detection of iodide formed. The optimal conditions for the separation and derivatization reaction were established by varying concentration of iodine, electrolyte pH and applied voltage. The optimised separations were carried out in 20 mmol l^{-1} Tris–HCl electrolyte (pH 8.5) using direct UV detection at 214 nm. Experimental results showed that the injection of the iodine zone from anodic end of the capillary gives significantly better precision. Common UV absorbing anions such as Br^-, I^-, $S_2O_3^{2-}$, NO_3^-, NO_2^-, SCN^- did not give any interferences. Valid calibration ($r^2=0.998$) is demonstrated in the range $1\cdot10^{-5}$–$8\cdot10^{-4}$ mol l^{-1} of sulfite. The detection limit ($S/N=3$) was $2\cdot10^{-6}$ mol l^{-1}. The proposed system was applied to the determination of free sulfite in wines. The recovery tests established for wine samples were within the range 92–103%. The CE results were compared with those obtained by iodometric titration technique.

Keywords: Derivatization, electrophoresis; In-capillary derivatization; Sulfite; Sulfur compounds; Iodine

1. Introduction

The determination of sulfite (or sulfur dioxide) is important in many environmental and industrial situations, particularly when monitoring atmosphere [1,2], foods and beverages [3,4], process liquors and wastewaters from paper mills [5], photographic laboratories [6,7] and mining sites [8].

Many analytical techniques are used to determine sulfite ions. Redox processes are generally involved in these determinations. The titration of sulfite with iodine best represents the classical approach to sulfite determination [9,10]. The simplicity of this method has significant limitations in terms of sensitivity and selectivity when dealing with authentic, real world samples. More sensitive spectrophotometric methods involve redox reactions with sulfite in which a coloured compound such as fuchsin [11,12] or Fe(II) complex with 1,10-phenantroline [13,14] is formed or decomposed. Again, the presence of other redox active species can cause significant interference in these systems through competing side reactions. In addition, spectrophotometric method would not be

*Corresponding author. Tel.: +370-2-336-310; fax: +370-2-330-987.

E-mail address: audrius.padarauskas@chf.vu.lt (A. Padarauskas).

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suitable for the analysis of highly coloured samples such as red wines and certain fruit juices.

Within the last decade capillary electrophoresis (CE) has become a versatile analytical technique employed for the determination of inorganic ions [15,16]. Most CE separations of inorganic ions are carried out using indirect UV detection because of the low UV absorptivities of these analytes [17]. This detection technique is however susceptible to interferences of matrix species and typically offer significantly less sensitivity when compared with direct UV detection. The chemical derivatization of the analyte leading to UV absorbing species presents a powerful strategy for improvement of the determination selectivity and detectability. There are two main approaches for accomplishing the derivatization of inorganic analytes in CE [18]: pre-capillary derivatization and on-capillary derivatization. In the first approach an excess of a reagent is added to the sample prior to injection, whereas on-capillary derivatization usually involves the addition of a reagent to the carrier electrolyte with subsequent reaction within the capillary. Both these techniques, however, are hardly useful for the analysis of real samples containing some species with similar chemical properties and, therefore, forming the same derivative.

Since CE is usually performed in free solution in the narrow capillary, a special section of the capillary can be reserved for derivatization reaction. Such in-capillary derivatization can be achieved by one of the following two techniques depending on where the reaction occurs. The first one [19–21] is the at-inlet technique, in which the sample and the reagent solutions are introduced to the inlet of the capillary either by tandem or sandwich mode, and these reactants are mixed by diffusion and allowed to react by standing the successive plugs for a specified period of time.

The second technique, the zone-passing, is based on derivatization in the middle of the capillary by passing either the sample or the reagent zone through the other during the electrophoretic separation. Taga et al. [22] successfully applied this method to the analysis of several amino acids using *o*-phthalaldehyde as the derivatization agent. This technique of derivatization seems very useful for selective determination of species present in complex matrices because before the derivatization the analyte (or analytes) is separated from other sample ions.

The main aim of this study was to evaluate the zone-passing technique for a simple and selective capillary electrophoretic determination of sulfite. The proposed system is based on the in-capillary derivatization of sulfite with iodine and direct UV detection of iodide formed.

2. Experimental

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

Deionized water was obtained by passing distilled water through a Milli-Q water-purification system (Millipore, Eschborn, Germany). Tris(hydroxymethyl)aminomethane (Tris) was purchased from Sigma (St. Louis, MO, USA). All other reagents were of analytical-reagent grade obtained from Merck (Darmstadt, Germany). All electrolyte and standard solutions were prepared using helium degassed deionized water. A stock sulfite solution (about 0.01 mol l^{-1}) was prepared daily by dissolving 0.126 g of Na_2SO_3 in 100 ml of oxygen-free water, and was standardised by iodometric titration.

The stock solution of iodine (0.01 mol l^{-1}) containing about 0.01 mol l^{-1} potassium iodide was standardised by titration with 0.01 mol l^{-1} sodium thiosulfate. Working sulfite and iodine solutions were prepared daily before use by suitable dilution.

All electrolyte solutions were filtered through a 0.2-μm membrane filter. The capillary was rinsed with 1.0 mol l^{-1} 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.

3. Results and discussion

3.1. Derivatization principle

The in-capillary derivatization reaction is carried out by mixing the analyte zone and the reagent zone during the electrophoretic separation. Such reaction should fulfil the following requirements: (a) the reaction should be fast; (b) the reaction should be quantitative or at least reproducible and (c) the molar absorptivity of the reaction product detected should be high to achieve high detection sensitivity. The most common approach to the analysis of sulfite is based on the titration with iodine according to the reaction [9,10]:

$$SO_3^{2-} + I_2 + H_2O \leftrightarrows SO_4^{2-} + 2I^- + 2H^+ \quad (1)$$

This reaction is fast and iodide formed strongly absorbs in the UV range (at 226 nm its molar absorptivity is 12 100 l mol^{-1} cm^{-1}) [23].

Fig. 1 shows the principle of the in-capillary derivatization procedure. The first step is the hydrodynamic injection of the iodine zone (Fig. 1a). Then the iodine zone is pushed towards the detector side by the injection of large volume of the electrolyte solution (Fig. 1b). The position of the iodine zone in the capillary can be varied by changing the injection time of the electrolyte. The third step is the injection of the sample solution (Fig. 1c). After the high voltage has been applied, the separation of the sample anions starts and the sulfite migrates towards the detector (Fig. 1d). The neutral I_2 zone migrates into opposite direction towards the cathode. During this step SO_3^{2-} ions are separated from other sample ions. The sulfite zone meets the I_2 zone and the reaction takes place. Then the I^- ions formed migrate towards the detector (Fig. 1e). This derivatization principle can be performed automatically using commercially available CE instrumentation. All the separations were performed under counter-electroosmotic conditions, in that electroosmotic flow (EOF) transports the electrolyte solution to the cathode, i.e., in the opposite direction to the migration of the analyte.

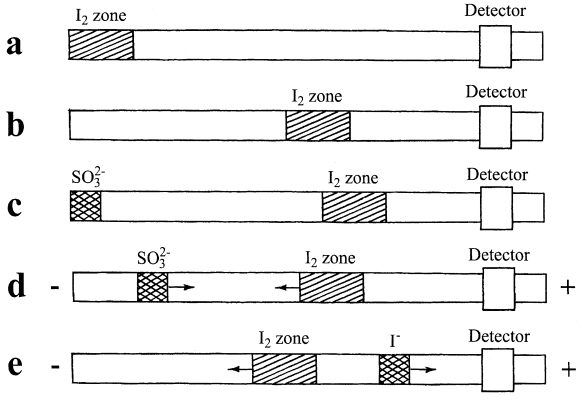

Fig. 1. Principle of the in-capillary derivatization procedure. (a) Injection of the iodine zone; (b) pushing of the iodine zone by electrolyte; (c) injection of the sample solution; (d) separation of the sulfite from other anions; (e) migration of the formed iodide towards detector.

3.2. Optimisation of CE conditions

For optimum peak shapes, the mobility of the electrolyte co-ion in CE must be as close as possible to the mobility of the analytes. In addition, buffering of the electrolyte is essential for reproducible and rugged separations. This factor should be especially important in the CE analysis of weak acid anions such as sulfite ($pK_{a2} = 7.2$). In order to obtain a high efficiency and pH stability with short analysis time the electrolyte pH and nature were therefore optimised.

In most cases for the iodometric titration of sulfite is used slightly acidic medium (pH 3–6). Therefore preliminary CE investigations were performed in the mixed acetate–sulfate electrolyte at pH 5.0. Fig. 2 shows the electropherogram obtained for a standard sulfite solution. Iodide, which is always present in the iodine solutions, appears as the first peak in the electropherogram. As can be seen, under these conditions sulfite gives very broad and poorly shaped peak. It is well known, that iodine reacts with iodide ions forming the soluble triiodide ion, I_3^- [24]:

$$I_2 + I^- \leftrightarrows I_3^- \quad (2)$$

Thus, I^- ions formed during the derivatization reaction can react with an excess of I_2 and, consequently, can cause peak broadening and/or partial

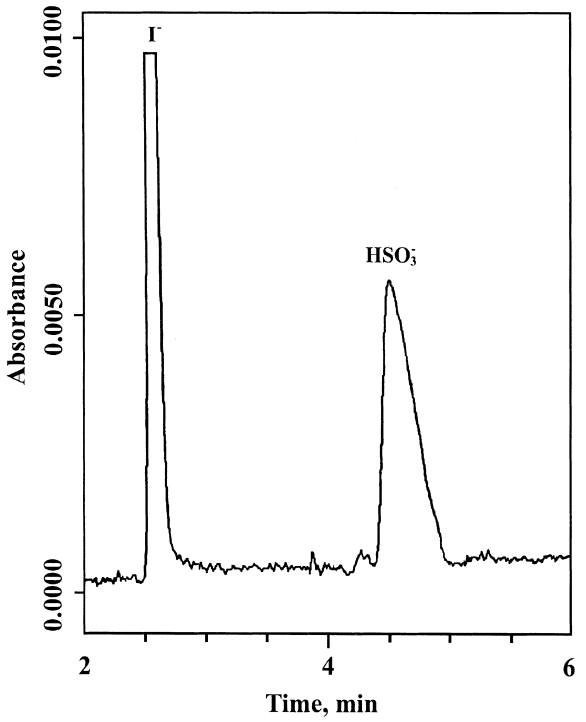

Fig. 2. Electropherogram of a standard ($2 \cdot 10^{-4}$ mol l^{-1}) sulfite solution. Electrolyte, 10 mmol l^{-1} Na_2SO_4, 2 mmol l^{-1} CH_3COONa, pH 5.0; injection, 10 s $5 \cdot 10^{-4}$ mol l^{-1} I_2, 100 s electrolyte, and 10 s sample; voltage, −30 kV; direct UV detection at 214 nm.

loss of the iodide formed. Several iodide standard solutions were therefore analysed using standard CE procedure and the proposed method with iodine zone. However, no significant differences in the migration times, peak areas and peak shapes for I^- ions were observed using both procedures indicating that equilibrium (2) does not influence the derivatization procedure.

Such peak broadening can be explained by the fact that the analyte and iodine zones are mixed gradually. At the first moment only a part of the sulfite react with iodine. Iodide formed migrates with higher mobility than still unreacted HSO_3^- and this difference in the mobilities causes peak broadening. Consequently, in the proposed system the maximum peak efficiency should be obtained in the case when mobility of the analyte is higher (or at least equal) than that of the detected reaction product. The mobility of weakly acidic sulfite increases with electrolyte pH. This effect is demonstrated in Fig. 3 in which the peaks obtained for the same sulfite concentration at different electrolyte pH are compared. As can be seen, the use of alkaline electrolyte gives much better efficiency.

Two different electrolyte co-ions – chloride (20 mmol l^{-1} NaCl electrolyte) and sulfate (10 mmol l^{-1} Na_2SO_4 electrolyte) – were compared and slightly sharper sulfite peak shapes using Cl^- co-ion were obtained. Finally, for the suppression of pH fluctuations during the separations Tris was added to the electrolyte as the counter-ion (instead of sodium) by neutralisation of 20 mmol l^{-1} HCl solution with Tris to pH 8.5.

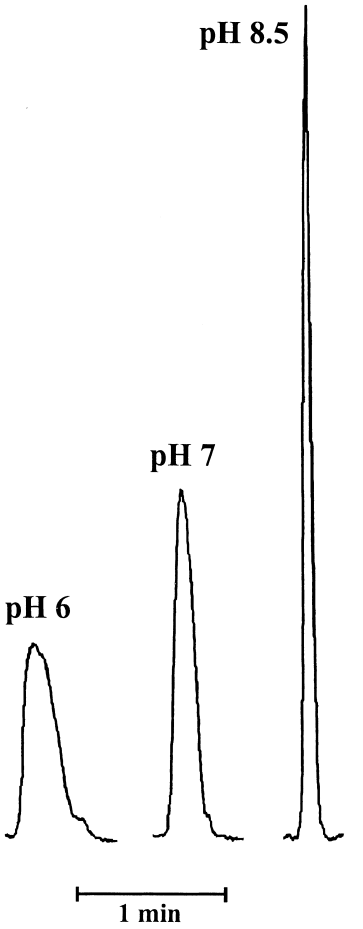

Fig. 3. Effect of electrolyte pH on the peak shape of the iodide formed. Electrolyte, 10 mmol l^{-1} Na_2SO_4; other conditions as in Fig. 2.

3.3. Effect of iodine concentration

The derivatization procedure described here requires quantitative or at least reproducible reaction between sulfite and iodine. Therefore, various concentrations of iodine were investigated in order to obtain a maximum peak area for sulfite standard. The iodine concentration was increased whereas the sulfite concentration was kept constant. The results obtained for three different sulfite concentrations are shown in Fig. 4. As can be seen, the area of the sulfite peak reaches maximum for $c(I_2)/c(SO_3^{2-}) \geq 1.2$. At lower $c(I_2)/c(SO_3^{2-})$ ratios, there is not enough iodine present for the quantitative derivatization.

Additionally similar experiments were performed in the 5 mmol l^{-1} NH_4NO_3 electrolyte at pH 8.5. In this case unreacted sulfite was monitored indirectly at 214 nm. No peak for sulfite was observed at $c(I_2)/c(SO_3^{2-}) \geq 1.2$.

The sulfite and iodine migration velocities and, consequently, the contact time of both zones depend on the voltage applied. At lower voltages both, sulfite and iodine zones migrate slower and their contact time increases. However, no changes in the time corrected sulfite peak area were observed when applying different high voltages in the range from 15 to 30 kV.

These results indicate that the derivatization reaction is complete under conditions used. In all further experiments 1 mmol l^{-1} iodine concentration was employed.

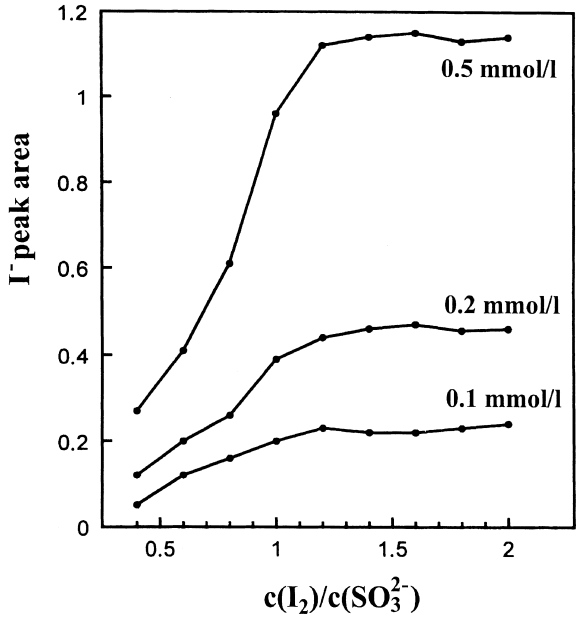

Fig. 4. Effect of iodine concentration on the peak area of the iodide formed for three sulfite concentrations. Electrolyte, 20 mmol l^{-1} Tris–HCl, pH 8.5; other conditions as in Fig. 2.

3.4. Determination of sulfite in real samples

At first, the effect of common UV absorbing anions on the determination of sulfite was studied. Anions such as Br^-, I^-, NO_3^-, NO_2^-, SCN^- did not give any interferencies at concentrations at least up to 2 mmol l^{-1}. Thiosulfate also reacts with iodine. Because thiosulfate migrates more rapidly than sulfite, a consumption of iodine by thiosulfate takes place and this causes interferences in the sulfite determination. When analysing samples with higher amounts of thiosulfate ions these interferencies can be eliminated by increasing iodine concentration. It should be noted that using the proposed system rapid simultaneous determination of common sulfur species such as sulfide, thiosulfate, sulfite, dithionite could be performed. These investigations are still in progress.

Fig. 5 shows the electropherogram obtained for a standard anion solution in the 20 mmol l^{-1} Tris–HCl electrolyte (pH 8.5). The separation selectivity can easily be improved by changing a position of the iodine zone in the capillary.

Several analytical performance characteristics important for quantitative analysis were measured. To determine the migration time and peak area repeatability, a solutions containing 0.1 mmol l^{-1} of sulfite were analysed sequentially six times. To avoid the repeatability test being effected by sulfite instability, a new, freshly diluted sulfite standard solution was injected for each run. The relative standard deviation (RSD) of the retention times was 0.5%, while the RSD of the peak areas was 8.9%. Such poor peak area repeatability probably is caused by the adsorption of iodine on the capillary wall. When the injected iodine zone is hydrodynamically pushed by the electrolyte, the desorption of adsorbed iodine occurs and after the concentrated iodine zone a very diluted and broad second iodine zone forms.

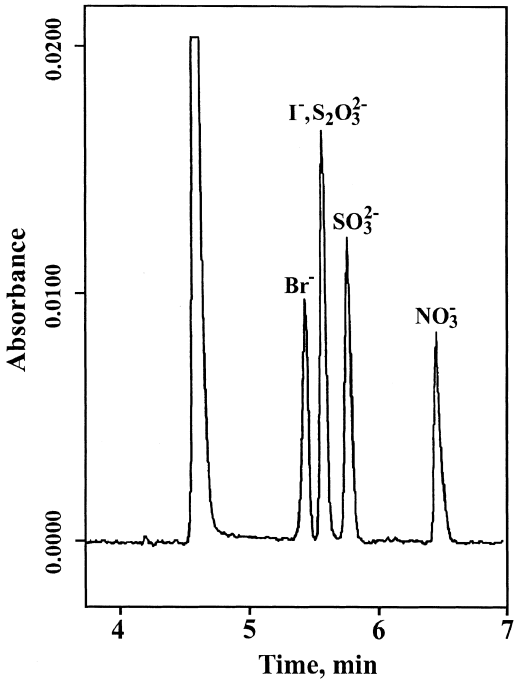

Fig. 5. Electropherogram of a standard solution of Br^-, I^-, $S_2O_3^{2-}$, SO_3^{2-} and NO_3^- anions. Voltage, −20 kV; other conditions as in Fig. 4.

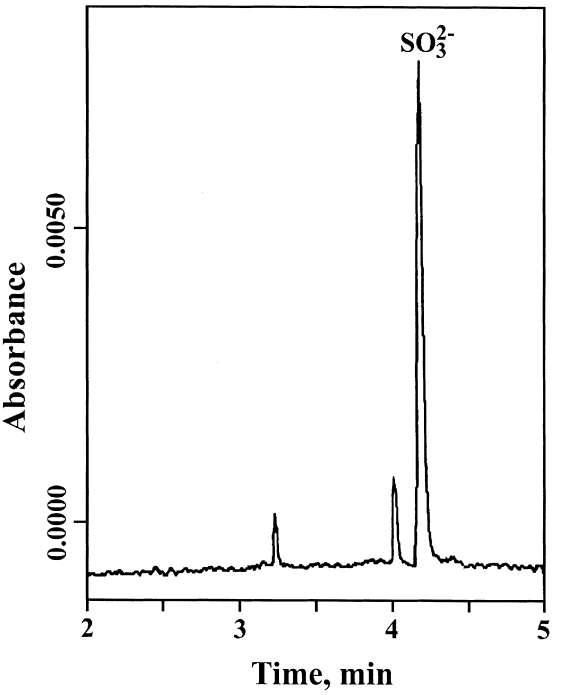

Fig. 6. Electropherogram of a sparkling apple wine sample. I_2 zone injected from anodic capillary end; other conditions as in Fig. 4.

This adsorption/desorption process is not reproducible. Depending on the iodine concentration in the diluted zone an appropriate amount of sulfite is oxidised before the sulfite reaches the first concentrated iodine zone. The peak area repeatability was significantly improved (2.8% RSD) by the injection of iodine zone from opposite (anodic) capillary end. In this case sulfite at first reaches the concentrated iodine zone and, consequently, its determination is not influenced by the diluted iodine zone.

The linearity of the calibration curve was measured by triplicate injections (10 s) of sulfite 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 $1 \cdot 10^{-5}$–$8 \cdot 10^{-4}$ mol l^{-1} of sulfite. It should be noted that upper limit of sulfite concentration in the calibration curve can be increased by increasing amount of the iodine solution injected. The detection limit determined for 20 s hydrodynamic injection was $2 \cdot 10^{-6}$ mol l^{-1} (three times the baseline noise).

To evaluate the proposed CE system for the real samples, it was applied to the determination of free sulfite in wines. Potential matrix interferencies were investigated by adding known amounts of sulfite ($5 \cdot 10^{-5}$–$5 \cdot 10^{-4}$ mol l^{-1}) to the sample solutions. The recoveries of the sulfite added to the wine samples ranged from 92 to 103%. The potential of the discussed system for the determination of sulfite in a real sample is shown in Fig. 6. Several wine samples were analysed by the CE method and by iodometric titration method [24]. The results are compared in Table 1. As can be seen, the results obtained are slightly lower in the iodometric method

Table 1
Data comparison (mg l^{-1} SO_2) of wine samples ($n = 3$)

Sample	CE	Titrimetry
White wine 1	28.0 (2.1)[a]	26.6 (1.6)
White wine 2	33.4 (2.2)	30.9 (1.2)
Sparkling apple wine (cider)	36.8 (1.8)	35.2 (1.5)
Red wine	6.2 (2.5)	–[b]

[a] Values in parentheses are RSD (%).
[b] Difficulties with the determination of the titration end-point.

than in CE. This difference is statistically significant, as determined by a *t*-test at a confidence level of 0.05. Additional recovery tests were therefore carried out for the iodometric method. An average recovery of 86% was obtained for white wine sample. Most probably this was due to partial loss of the analyte through volatilisation and/or aerial oxidation, that occur more rapidly under acidic conditions used for direct iodometric titration of sulfite in foods, as has been reported in a number of reviewed publications [25,26].

The analysis does not require any preliminary treatment of the samples except dilution. The proposed CE method appears to be a good alternative to iodometric and colorimetric procedures for determining sulfite in samples containing interfering substances.

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