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Determination of sulfide in the leather industry by capillary electrophoresis

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Abstract

Capillary electrophoresis (CE) was investigated for the determination of sulfide in effluents and waste water samples from the leather industry. A 10 m*M* sodium sulfate electrolyte at pH 10.5 was used and a quaternary ammonium hydroxide was added for the reversal of the electroosmotic flow (EOF). The linearity, separation efficiency and the effects of pH, concentration of the EOF modifier and the capillary dimensions were investigated. Direct UV detection at 229 nm provided the best specificity and also the best sensitivity. The limits of detection were 41 and 10 μg of S^{2-}/l using a 75- and a 100-μm I.D. capillary, respectively. The reproducibility was considerably improved by using the internal standard technique. Molybdate, iodide and biphthalate may be used as internal standards. Spiking of real samples gave recoveries between 102 and 111%. The CE method presented here was compared with the classical methods and it was concluded that it may be a good alternative for determining sulfide in difficult samples.

Keywords: Waste water; Water analysis; Leather; Sulfide; Inorganic anions

1. Introduction

Sodium sulfide is the most widely used unhairing agent in the leather industry. About 1–3% of technical grade sodium sulfide, calculated on the rawhide weight basis, is spent in this process [1]. Effluents of the unhairing process contain 700 to 2000 ppm of sulfide ion, and deliming effluents have 10 to 200 ppm. A typical untreated tannery waste water sample has a sulfide concentration of up to 20 ppm.

The determination of sulfides has been dominated by iodometric and methylene blue colorimetric methods [2]. The iodometric method is based on the reaction of iodine with sulfide, oxidizing it to sulfur. It is suitable for analyzing samples of high sulfide concentration. However, the occurrence of some other components that are capable of reducing iodine (like thiosulfate, sulfite and various organic compounds) may cause a positive bias. The colorimetric method is the most widely used in waste water analysis. Although it is very sensitive, in some leather waste water samples, this method suffers from matrix interferences that mask the development of methylene blue colour, with turbidities or foreign colours. Sulfide itself prevents the reaction if its concentration is very high, in the range of several hundred ppm, and this gives false negative results.

These methods, and others that have been used in the leather industry [3], have a lack of specificity.

Ion chromatography (IC) with electrochemical

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detection was succesfully used by Rocklin and Johnson [4] for the determination of sulfide ions. Cyanide and sulfide could be separated from the common anions and determined simultaneously. IC, both with electrochemical and with UV detection, has become a common method for determining sulfide in process liquors for the pulp and paper industry [5]. Nevertheless, IC has not been applied to the determination of sulfide in leather samples.

Capillary electrophoresis (CE) is a powerful separation technique. Its selectivity is based on the ionic equivalent conductances of the analytes and its separation efficiency typically exceeds 100 000 theoretical plates [6]. Salomon and Romano [7,8] have determined sulfide in samples from the pulp and paper industry by CE. Indirect (185 nm) and direct UV detection (214 nm) was applied to monitor sulfide and other anions, using a chromate electrolyte with an additive (OFM anion-BT from Waters) for the reversal of the electroosmotic flow (EOF).

Nevertheless, application of the procedure of Salomon and Romano to leather waste water samples is not always suitable because of a lack of sensitivity. In these samples, as well as in many deliming effluents, the concentration of chloride and sulfate may exceed the concentration of sulfide by a factor of 500. This excessive sulfate may mask the proper identification and quantitation of sulfide. A method with enhanced sensitivity should allow the introduction of more diluted samples into the capillary, which in turn, may allow the reduction of possible matrix effects.

This paper presents a method for determining sulfide with high specificity and improved sensitivity, based on CE with direct UV detection at 229 nm. At this wavelength, chloride and sulfate do not interfere with detection. A negative polarity is applied and the EOF is reversed. This approach significantly reduces the migration time of sulfide, which allows its determination in less than 4 min. A sodium sulfate electrolyte is used, and the EOF modifier is in the hydroxide form, instead of the usual bromide salt. The removal of bromide anion increases the sensitivity of direct detection in the 185–229 nm range. Another advantage is the prevention of the bromide peak generated as a result of the reversed electrostacking phenomenon that occurs when samples have higher conductivities than the electrolytes [9].

2. Experimental

2.1. Instrumentation

The CE instrument used was a Waters capillary ion electrophoresis (CIE; Waters' trade name Capillary Ion Analyzer) system, equipped with a negative power supply and a Cd lamp with a 229 nm optical filter. A Hg lamp for detection at 185 and 254 nm and a Zn lamp for detection at 214 nm were also used. The applied voltage was −20 kV. Separations were carried out using fused-silica capillaries of 60 cm×75 μm I.D. In order to improve sensitivity, a capillary of the same length but with an I.D. of 100 μm was tested. Both capillaries were obtained from Waters (AccuSep). Samples were introduced by a 30-s hydrostatic injection from a height of 10 cm. The external temperature of the capillary was adjusted to 25°C. The detector time constant was set at 0.1 s. Data acquisition was carried out with a Waters Millenium 2010 chromatography manager with a SAT/IN module connecting the CE to the data station.

Every morning, the capillary was washed for 2 min with 1 *M* NaOH, for 3 min with water obtained from a Milli-Q system (Millipore) and for 5 min with the working electrolyte. Between runs, the capillary was purged for 2 min with electrolyte. At the end of the working session, the capillary was rinsed with Milli-Q water for 5 min and left in water.

2.2. Reagents and standards

All solutions were prepared in water obtained from a Milli-Q water purification system. Sulfide standards were prepared freshly from $Na_2S{\cdot}9H_2O$ (ACS-grade), obtained from Sigma. Solid sodium sulfide was stored in a desiccator at 4°C. Ethylenediamine was supplied by Sigma. All other standards (sodium thiosulfate, potassium iodide, potassium nitrate, ammonium molybdate and potassium biphthalate), and reagents (ascorbic acid, H_2SO_4 and NaOH) were of analytical grade and were purchased

from Panreac (Barcelona, Spain) and used without further purification.

2.3. Preparation of working electrolyte

The working electrolyte consisted of 10 m*M* Na_2SO_4 and 0.5 m*M* OFM–OH, pH 10.5±0.1 prepared from a stock standard solution of 200 m*M* Na_2SO_4 (Sigma, ACS grade) and a 20 m*M* concentrate of OFM–OH obtained from Waters (CIA Pak OFM anion–OH). This reagent is an alkylammonium salt in the hydroxide form and it is an osmotic flow modifier (OFM) for the reversal of the direction of the EOF. The electrolytes were prepared fresh daily, degassed prior to use, and they were refreshed after 2–3 h of electrophoresis.

2.4. Samples

Samples were collected at local tannery factories. Flared mouth flasks were used for sampling. The flasks were filled completely to the top without any air. Samples were stored refrigerated and were analyzed within 2 h. The only clean-up procedure used was microfiltration through a 0.45-μm Millex HV filter (Millipore). Samples were diluted to a sulfide concentration within the range 1 to 5 mg/l and mixed with an internal standard. The pH of diluted samples was kept highly alkaline by the addition of 4 ml of 1 *M* NaOH per litre of solution prior to adjusting the final volume. Samples were injected immediately after dilution.

2.5. Calibration

Five standards within the range 0.5 to 10 mg/l of S^{2-} were prepared. Every standard contained 4 ml of 1 *M* NaOH per litre of solution. Iodide (10 mg/l of I^-), molybdate (20 mg/l of $Mo_7O_{24}^{6-}$), or biphtalate (10 mg/l of $C_8H_5O_4^-$) were used as internal standards. Standards were prepared and injected immediately. Calibration graphs were plotted based on the linear regression analysis, without forcing the curve through the zero.

3. Results and discussion

3.1. Detection

The UV spectrum of sodium sulfide shows an absorption maximum at 229–230 nm and a considerable absorption at wavelengths near 185 nm. However, the largest analyte signal does not necessarily imply the highest sensitivity, since a low baseline noise is also needed. Higher lamp energies provide lower baseline noise [10]. The limit of detection (LOD) is defined as the amount of analyte that would give a signal three times that of the baseline noise. The LOD of sulfide was measured at four different wavelengths, using the fixed-wavelength detector mounted in the CIE system. This kind of detector offers higher sensitivity in the range between 185 and 254 nm than offered by the variable-wavelength instruments [11]. Results are given in Table 1. From this data, it can be seen that the lower LOD of sulfide is at 229 nm, although the largest signal is at 185 nm.

Detection limits were also determined using the chromate electrolyte described in Refs. [7,8]. The lowest LOD obtained was at the wavelength of 214 nm and it was averaged to 200 μg of S^{2-}/l. Therefore, the LODs at 229 nm detailed in Table 1

Table 1
Limit of detection (LOD) of sulfide in μg of S^{2-}/l and R.S.D. (%) (in brackets), at four different wavelengths using two fused-silica capillaries

	LOD (μg S^{2-}/l)			
	185 nm	214 nm	229 nm	254 nm
Capillary of 75 μm I.D.	83 (16)	184 (19)	41 (12)	104 (20)
Capillary of 100 μm I.D.	22 (10)	48 (12)	10 (7)	29 (12)

Dimensions of the capillaries are 60 cm×75 μm I.D. and 60 cm×100 μm I.D., respectively. The electrolyte is 10 m*M* sodium sulfate–0.5 m*M* OFM–OH, adjusted to pH 10.5. The injection was hydrostatic (10 cm for 30 s). Reported LODs are the average of the data obtained from six injections made on three different working days.

resulted in approximately a 5-fold improvement in sensitivity (20 times if the 100 μm I.D. capillary is used) when compared to the chromate electrolyte and 214 nm detection.

Direct detection at 229 nm may provide good sensitivity and at the same time an improved specificity compared to indirect UV detection. Only a very short list of inorganic anions have strong absorbance at this wavelength. Iodide and molybdate anions have considerable absorption at 229 nm. Therefore, they could be used as internal standards, since they are not present in leather samples. Orthophthalate is an alternative internal standard, but its higher migration time increases the length of the analysis (Fig. 1). Low concentrations of thiosulfate, nitrite and nitrate are found in some samples but they do not cause interferences since their peaks are well resolved from sulfide. As can be seen from Fig. 2, all of these 229 nm-absorbing anions can be monitored in the same run as sulfide.

3.2. Influence of electrolyte pH on selectivity

The effect of the pH of the electrolyte on selectivity was investigated. Sulfide and five other anions that absorb at 229 nm were evaluated with varying pHs from 7.5, rising in increments of 0.5 pH units, to 12.5. The pH was adjusted by the addition of 100 m*M* H_2SO_4 or 100 m*M* NaOH, depending on the selected pH. Fig. 3 shows that no changes in selectivity occur between pH 7.5 and 11.5. There is no significant pH dependence of the tested ions at a pH of below 11. Sulfide normalized migration times are nearly constant in the pH 7.5 to 11.5 range. This is explained by the fact that the predominant form of sulfide in this range is HS^-. Above pH 12, the

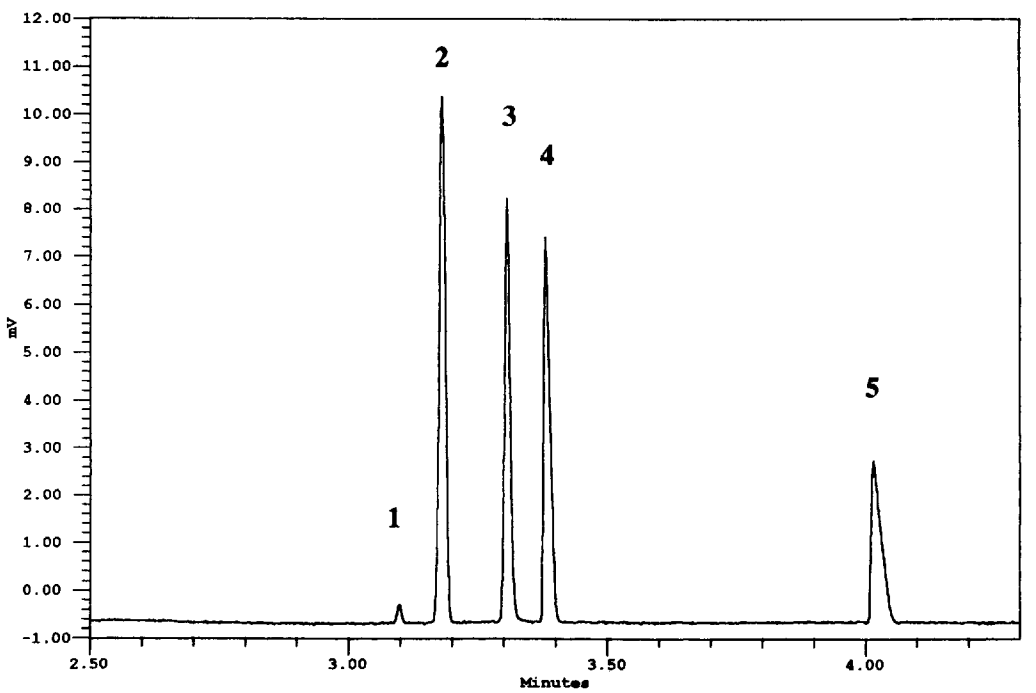

Fig. 1. Electropherogram of an unhairing effluent sample with the addition of three substances that may be used as internal standards. Capillary, fused-silica, 60 cm×75 μm. Direct UV detection was performed with a Cd lamp at 229 nm. See Section 2 for more details. The sample was filtered, diluted 1:300, and the pH was adjusted to 11.4 by the addition of NaOH. Peaks: 1=thiosulfate (not quantitated); 2=iodide (I.S.); 3=sulfide, 1010 ppm; 4=molybdate (I.S.); 5=biphthalate (I.S.). The concentrations of internal standards in the diluted sample are 10 mg/l of I^-, 20 mg/l of $Mo_7O_{24}^{6-}$ and 10 mg/l of $C_8H_5O^{4-}$.

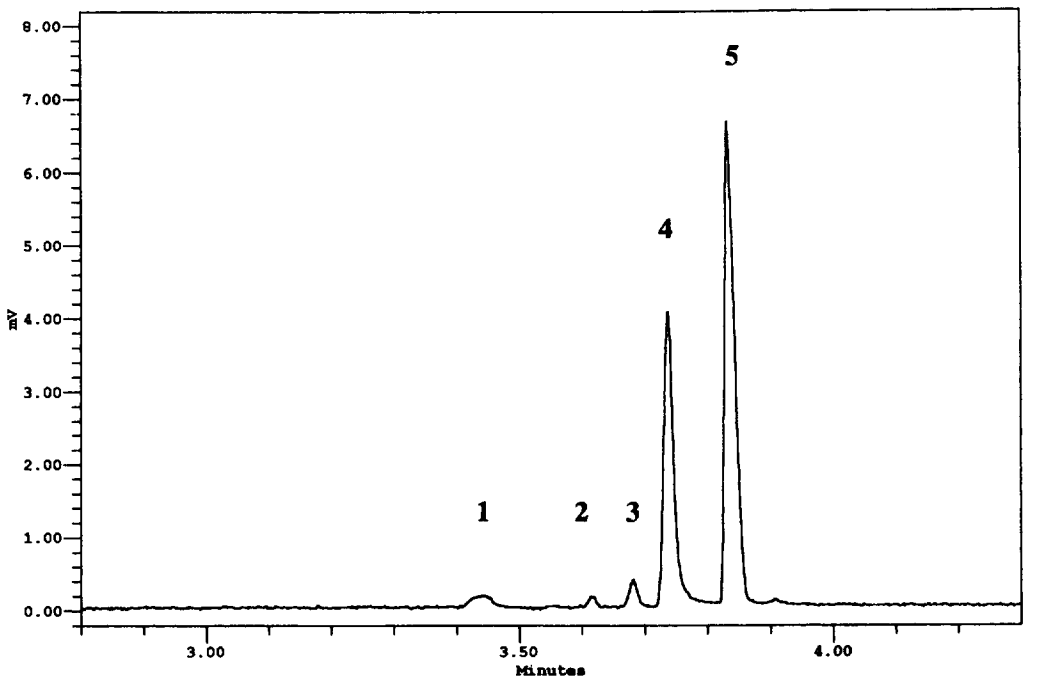

Fig. 2. Electropherogram of waste water sample. For experimental conditions, see Fig. 1. The sample was filtered, diluted 1:8 and the pH was adjusted to 11.4 by the addition of NaOH. Peaks: 1=thiosulfate (not quantitated); 2=nitrite (not quantitated); 3=nitrate, 16 ppm; 4=sulfide, 19.2 ppm; 5=molybdate (internal standard). This sample also contained chloride (800 ppm) and sulfate (900 ppm) but these anions are not detected at 229 nm.

mobility of sulfide slightly increases, because it is near to its second pK_a. A fast decrease in migration time is observed for biphthalate above pH 11. This was quite predictable because biphthalate approaches its second pK_a, becoming phthalate. Good resolution is observed at every pH below 11. pH 10.5 was chosen for the working electrolyte because of the higher stability of sulfide in alkaline conditions. In addition, it is very similar to the pH of real diluted samples.

3.3. Separation efficiency

From Fig. 1 and Fig. 2, it can be seen that the peaks of sulfide and the internal standards are well resolved using a capillary of 75 μm I.D. The number of theoretical plates was calculated for the sulfide peak injecting standards of 0.5 ppm of S^{2-}. The average result was 100 500 for the 75 μm capillary. However, resolution is lower when the 100 μm I.D. capillary is used instead.

3.4. Time stability

The sulfide anion is unstable. In the presence of air it may be oxidized to sulfur, thiosulfate or sulfate, depending on the excess of oxygen. Loss of sulfide may occur from escaping volatile hydrogen sulfide (H_2S), at pH values of less than 9. Both decomposition processes are expected to be delayed in concentrated samples, like deliming and unhairing effluents, by completely filling the bottles and leaving no air space. These samples are alkaline (pH values up to 12.5) and they have an antioxidant matrix that contains thiosulfate, sulfite, and organic compounds like aldehydes, carbohydrates and amino acids, obtained from collagen and keratin degradation. Dissolved oxygen (DO) was measured in five of these

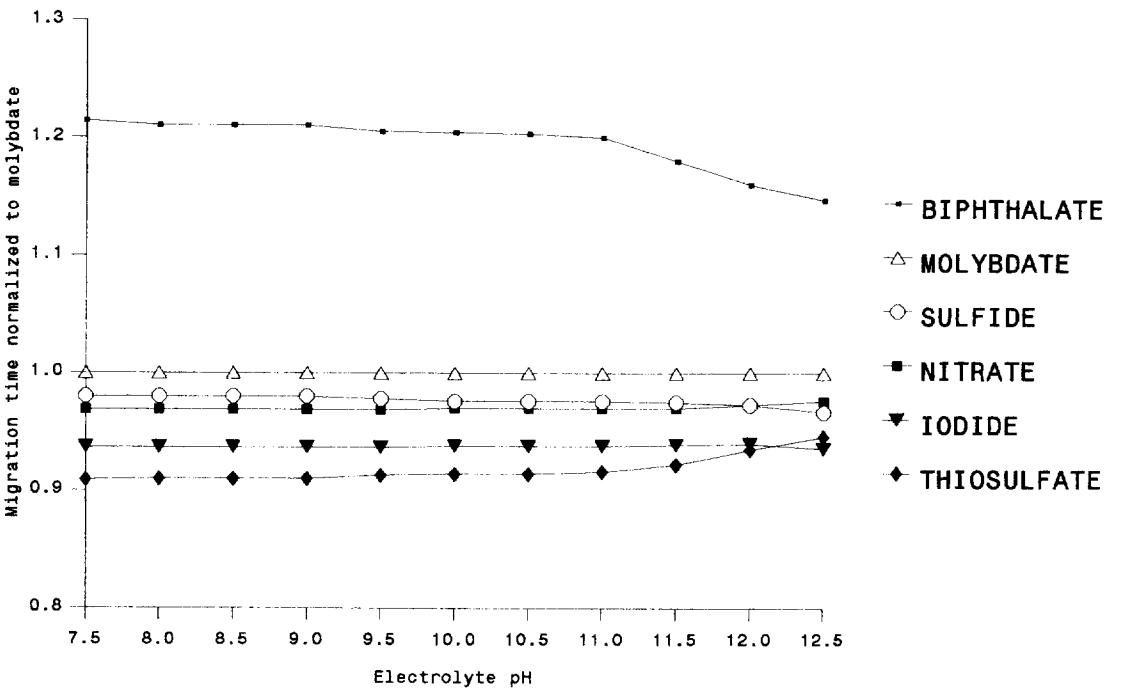

Fig. 3. The normalized migration times of sulfide and five other anions that absorb at 229 nm are plotted against the pH of the electrolyte, 10 m*M* sodium sulfate–0.5 m*M* OFM–OH. Precision: The R.S.D.s of normalized migration time for triplicate injections were between 0.05 and 0.10%.

samples by the membrane-electrode technique. In all cases, DO was found to be 0.0 mg/l. To test the stability of concentrated samples, a fresh sample of an unhairing effluent was distributed between eight bottles, that were filled completely to the top. They were stored refrigerated and sequentially analyzed at intervals of about 45 min. The electrolyte was refreshed every 2 h of working. Sulfide decomposition was negligible for 2 h and it only reached the 1% level after 6 h.

However, standards and diluted samples do not have the protection provided by the antioxidant matrix. One might reasonably suppose that the oxidation rate will be faster than in concentrated samples. In the analysis of pulping liquors by IC, 1 ml/l of an antioxidant buffer (176 g of ascorbic acid, 40 g of NaOH and 10 ml of ethylenediamine in 1000 ml of water) is added to standards and diluted samples to combat oxidative loss of sulfide [5]. The effect of the antioxidant buffer on the time stability was investigated. A set of standards and samples, diluted to approximately 2.5 mg S^{2-}/l, with and without antioxidant, were analyzed as a function of storage time. The results had relatively high variations. Sulfide decomposition was found to range from 0 to 5% at 20 min, from 2 to 7% at 40 min and from 2 to 9% at 60 min. Unexpectedly, the solutions with antioxidant did not show a significant improvement on stability. Moreover, a new small peak following the sulfide is observed in these solutions. This peak is badly resolved and the integration of the sulfide becomes more difficult.

The rapidity of the CE technique allows the recording of the sulfide peak in less than 5 min after the preparation of the solution, including the time needed for filling the vial and injecting. It was concluded that if samples and standards are prepared and injected immediately, the influence of oxidation should be minimal.

3.5. Linearity

The linearity of the calibration plots in the range 0.5–10 ppm of sulfide was determined. Linearity at

higher concentrations was not investigated. Both calibration methods (external and internal standard) gave similar, good results, with typical correlation coefficients of 0.9999. No significant differences were obtained for iodide, molybdate, or biphthalate as internal standards.

3.6. Repeatability and reproducibility

To determine the repeatability of peak area and migration time, a sample containing 3.6 ppm of sulfide and 20 ppm of molybdate, adjusted to pH 11.4 with 1 *M* NaOH, was analyzed sequentially eight times on a single day. To avoid the repeatability test being effected by sulfide instability, a new, freshly prepared, sample was injected for each run. Between runs, the capillary was purged for 2 min with electrolyte. The relative standard deviation (R.S.D.) of sulfide peak areas was 8.0%, while the R.S.D. of sulfide area normalized to the area of molybdate was only 0.7%. The reproducibility was determined by injecting a freshly prepared solution of the same composition over seven working days and calculating the R.S.Ds. The reproducibility obtained using normalized areas was considerably better (2.1% R.S.D.) than that found using uncorrected areas (7.0% R.S.D.)

The use of iodide and biphthalate as internal standards, instead of molybdate, gave results with somewhat lower precision.

The repeatability and reproducibility of sulfide migration time was also much higher for normalized data (0.04 and 0.09% R.S.D., respectively) than for uncorrected data (1.4 and 2.1% R.S.D., respectively). The pH of the electrolyte shifts to lower values as a result of diffusion of carbon dioxide from the atmosphere. Changes in the pH of the electrolyte, as well as variations in the concentration of the samples, and changes in the capillary wall, affect the velocity of the EOF which, in turn, influences the analyte migration times. Fortunately, sulfide and molybdate are influenced in a similar way. Therefore, normalization of sulfide migration times to molybdate significantly improves the precision of results.

Alkylammonium ions like OFM are known for their ion-pairing ability [6]. Therefore, it would be expected that the OFM at a concentration of 0.5 m*M* might be overloaded in samples with high concentrations of anions. This would imply a decrease in OFM mobility and, in turn, changes in sulfide migration times. The stability of migration times was examined by injecting a standard of sulfide and molybdate, prepared using increasing concentrations of sulfate over the range 0–225 ppm and using the OFM at a concentration of 0.5 m*M*. It was found that the sulfide migration times normalized to molybdate were unaffected by the increasing sulfate concentration. The same results were obtained using chloride instead of sulfate.

By varying the concentration of the OFM within a range from 0.5 to 3.0 m*M*, the migration order shown in Fig. 1 and Fig. 2 gradually changed to sulfide – nitrate – thiosulfate – molybdate – iodide – biphthalate. Nevertheless, the best resolution is achieved at 0.5 m*M* OFM.

In conclusion, no reasons were found for increasing the OFM concentration.

3.7. Accuracy

A sample of beamhouse waste water was analyzed by the CE and the colorimetric methods. This sample was an alkaline mixture of effluents and washings from the industrial processes of soaking, unhairing and deliming. The sample was filtered and diluted 1:25. Molybdate was added as the internal standard prior to adjusting the final volume. Eight diluted samples were sequentially prepared and injected immediately. All these solutions were adjusted to pH 11.3–11.5 by the addition of the same amount of 1 *M* NaOH (see Section 2). The same sample was simultaneously analyzed by the methylene blue colorimetric method [2]. Sample preparation included filtration and dilution (1:125). The average results of the eight determinations were 113.3 and 112.4 mg of S^{2-}/l using CE and colorimetry, respectively. Very good agreement between the two methods was observed. CE gave improved precision, with a R.S.D. of 1.3% compared to 3.4% for the colorimetric technique.

A sample of unhairing effluent was simultaneously analyzed by both CE and iodometric methods [2]. The sample was filtered and was diluted 400-fold in CE, whereas no dilution was made in the volumetric method. Molybdate was used as the internal standard

in CE. The results obtained from eight determinations were higher in the iodometric method (average 943.5 mg of S^{2-}/l) than in CE (average 873.3 mg of S^{2-}/l). This difference was statistically significant, as determined by a *t*-test at a confidence level of 95%. The iodometric method probably suffers the interference from other compounds present in this sample, like thiosulfate or aldehydes, which reduce iodine as well as sulfide.

Two aliquots of a sample of deliming effluent that contained 195 mg of S^{2-}/l were spiked with sulfide up to 270 and 346 mg of S^{2-}/l, respectively, and they were immediately analyzed by CE. An average recovery of 105% was obtained for each spiked sample. Additional recovery tests were carried out with two unhairing effluents of approximately 1000 mg of S^{2-}/l spiked with 250 and 500 mg of S^{2-}/l, respectively. The calculated recoveries ranged from 102 to 111%.

4. Conclusions

CE with direct detection at 229 nm provides a rapid, sensitive and highly specific method for determining sulfide in leather samples. Sample preparation is as simple as filtration, dilution and addition of internal standard. CE appears to be an excellent alternative to classical procedures for determining sulfide in samples containing interfering substances that may give inaccurate or false negative results, by the iodometric and the colorimetric methods.

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