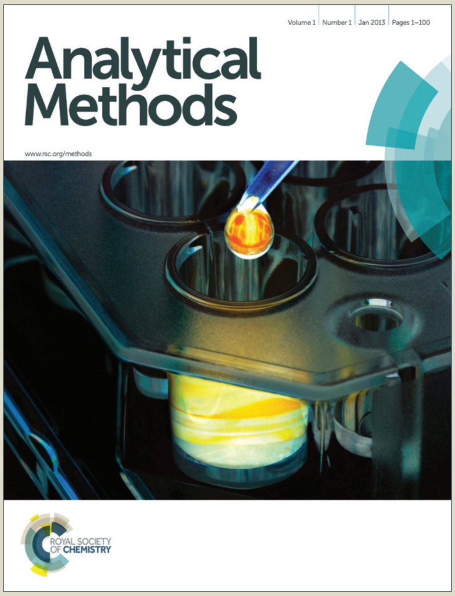
# Analytical Methods



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# Systematic optimization of a pyromellitic acid background electrolyte for capillary electrophoresis with indirect UV-Vis detection and online pre-concentration analysis of thiosalt anions in the treated mine tailings

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## Abstract

A CZE method using indirect UV-vis detection was developed for the simultaneous separation and determination of five thiosalt anions: sulfate ($SO_4^{2-}$), thiosulfate ($S_2O_3^{2-}$), trithionate ($S_3O_6^{2-}$), tetrathionate ($S_4O_6^{2-}$) and pentathionate ($S_5O_6^{2-}$). As with many inorganic anions, absorbance by some thiosalt species in the UV-Vis range is poor or non-existent. Fully ionized pyromellitate ion (PMA), with a high molar absorptivity was evaluated as the chromophoric probe for analysis of these species. The factors considered in optimization of method were: composition of the background electrolyte (BGE) (concentrations of PMA and electroosmotic flow (EOF) modifiers, pH of BGE, applied electric potential (V) and plug size of water for online sample pre-concentration. The optimal conditions were: [PMA] = 2.00 mM, [hexamethonium ($HM^{2+}$)] = 0.80 mM, pH = 8.0, E = -30 kV and online sample pre-concentration (stacking) with 90 mbar.s injection of water followed by 250 mbar.s injection of thiosalt standard sample before application of separation voltage. The five thiosalt anions were separated in under 3 min with good resolution and sensitivity obtained for all the analyte peaks. Limits of detection (LOD) were between 0.09 and 0.34 µg/mL without stacking; a three-fold increase in LODs to between 0.02 and 0.12 µg/mL was achieved after online pre-concentration.

Keywords: thiosalts, chromophoric probe, EOF modifiers, sample stacking

## 1. Introduction

Thiosalts are sulfur oxyanions formed during the incomplete oxidation of sulfur-rich minerals. Most thiosalt species are produced during the milling and flotation of these sulfidic ores, particularly those containing pyrite ($FeS_2$) and pyrrhotite ($Fe_{(1-x)}S$, x = 0 to 0.2).[1] The major thiosalt species are sulfate ($SO_4^{2-}$), thiosulfate ($S_2O_3^{2-}$), trithionate ($S_3O_6^{2-}$), tetrathionate ($S_4O_6^{2-}$) and higher polythionates such as pentathionate ($S_5O_6^{2-}$). Thiosalts can be oxidized to sulfate, a process that is accompanied by the production of acid, which can lead to acidification of receiving waters, which includes rivers, lakes, and the ocean into which the treated tailings are discharged.[2] Tailings ponds or mine dumps containing waste are typically treated by chemical oxidation with hydrogen peroxide/ferric sulfate or biochemically, and further neutralized by lime treatment. Although thiosalts have been shown to be relatively non-toxic, acidification can cause stress to freshwater flora and fauna, which in some instances can be severe enough to lead to death of vulnerable organisms. Acidification can also result in enhanced metal migration and toxic metal concentrations.[1,2]

Though the discharge of thiosalts is not directly regulated, acid mine drainage and the increased solubility of toxic metals are a direct consequence of thiosalts oxidation. Thus, the effective mitigation and treatment of thiosalts present a significant challenge to both the mining and metals processing industries.[3] Analysis of thiosalts in milling process waters and tailings ponds is necessary for effective treatment and the data is also needed to develop a better understanding of how thiosalts are generated, which in turn informs the treatment protocols. Despite extensive research in the area since the 1980s, there are still many unknowns, specifically questions related to reaction kinetics and thermodynamics of thiosalts transformations, which are complex and interrelated.[2-4] To study thiosalts reaction kinetics, which can show significant changes on a time-scale of minutes, improved method of analysis are required.[4,5] Ion chromatography (IC) coupled with various detection techniques has been used generally for their determination and quantitation.[5] IC is a well-established technique with numerous well-developed methodologies for the determination of inorganic anions with good measures of reliability and sensitivity.[5-10] However, consumables can be costly, separations have only moderate resolution and analyses tend to be time consuming. In addition, samples with high ionic strength may give less efficient separations for trace analyte concentrations and limit the use of non-spectral detection methods. Capillary zone electrophoresis (CZE) has inherent advantages, such as fast analyses, tolerance for a range of sample matrices, low consumption of reagents, and simple instrumentation, making it an attractive alternative to IC. [6-11]

A substantial amount of analytical work[5, 13-18] has been done in the area of quantitation of sulfur anions in water, air and soil samples. Table 1 summarizes some of the methods published for sulfur anions by CZE with indirect UV-vis detection. As exemplified in the table, the most common chromophoric probes for the analysis of sulfur oxyanion species in CZE include pyromellitic acid (PMA) and sodium chromate ($Na_2CrO_4$). PMA, also known as benzene-1,2,4,5-tetracarboxylic acid is a high UV-absorbing molecule. Its fully ionized form (pyromellitate) was evaluated in this work as the BGE probe.

Electroosmotic flow (EOF) modifiers, most often organic solvents and surfactants like hexadecyltrimethylammonium bromide (CTAB), tetradecyltrimethyl-ammonium hydroxide (TTAOH) and diethylenetriamine (DETA), are added to the background electrolyte (BGE) solution (or buffer solution used for the analysis) to change the magnitude or direction of EOF through modification of the chemistry of the capillary surface. From these applications, it can be concluded that the most important factors influencing separation efficiency of CZE with indirect detection are concentrations of chromophoric probe and EOF modifier, pH, capillary temperature, and applied electric field.

Table 1. Overview of indirect CZE applications for the analysis inorganic sulfur anions and the limit of detection range (of minimum and maximum LOD values) (μg/mL)

| **BGE system** | **BGE additives** | **Sulfur species analyzed** | **LOD range (μg/mL)** | **Reference** |
|---|---|---|---|---|
| 1.5 mM PMA, 10 mM Tris; pH 8.0, $\lambda = 214$ nm | 0.5 mM CTAB | $S_2O_3^{2-}$, $S_2O_3^{2-}$, $SCN^-$, $SO_3^{2-}$ | 0.17 - 0.50 | [7] |
| 5 mM $Na_2CrO_4$, pH 9.4, $\lambda = 374$ nm | 4 mM TTAOH,<br>10 mM 2-cyclo-hexylaminoethanesulfonic acid (anti-coagulant) | $SO_4^{2-}$, $SO_3^{2-}$, $S_2O_3^{2-}$, $S_2O_4^{2-}$ | 0.45 - 0.48 | [14] |
| 2.25 mM PMA, 6.5 mM NaOH, 1.6 mM TEA; pH 10, $\lambda = 254$ nm | 0.75 mM HMOH | $S^{2-}$, $SO_4^{2-}$, $SO_3^{2-}$, $SCN^-$, $S_2O_3^{2-}$, $S_2O_6^{2-}$, $S_2O_8^{2-}$ | 0.35 - 0.35, | [18] |
| 10 mM $Na_2CrO_4$, pH 11, $\lambda = 275$ nm | 2 mM TTAOH | $S^{2-}$, $SO_4^{2-}$, $SO_3^{2-}$, $S_2O_3^{2-}$ | N/A | [19] |
| 1.5 mM PMA, 10 mM Tris; pH 7.0, $\lambda = 214$ nm | 0.5 mM DETA,<br>0.1% formaldehyde (stabilizer) | $SO_4^{2-}$, $SO_3^{2-}$, $S_2O_3^{2-}$, $S_2O_4^{2-}$ | 0.45 - 1.0 | [20] |

N/A – not reported

In addition to optimizing instrument and BGE parameters, there have been several reports on improving the detection sensitivity of CZE using on-line pre-concentration sample stacking techniques such as field-amplified sample stacking[19,20], large volume sample stacking[21,22] and pH-mediated sample stacking[23,24]. These techniques work on the principle of introducing a plug of the sample at low ionic strength relative to the BGE into a BGE-filled capillary and applying a high potential. The high electric field experienced by the analytes in the sample plug causes them to move rapidly until they reach the BGE interface at which point the analytes slow down and ‘stack’ leading to a concentrated zone of analytes and increased sensitivity.

In this work, we report the systematic development of a CZE with indirect UV-vis method with PMA as the chromophoric probe and three EOF modifiers: tetramethylammonium hydroxide (TMAOH), CTAB and hexamethonium hydroxide (HMOH). The BGE was further optimized by varying the most important factors: concentrations of chromophoric probe and EOF modifier, pH and applied field voltage, for the fast separation (under 3 min) of five key thiosalts species (sulfate ($SO_4^{2-}$), thiosulfate ($S_2O_3^{2-}$), trithionate ($S_3O_6^{2-}$), tetrathionate ($S_4O_6^{2-}$) and pentathionate ($S_5O_6^{2-}$), which have not been analysed together by CZE previously. The optimized BGE was compared with a costly commercially available PMA BGE solution, and it was found to provide much better sensitivity and complete separation of the five species, which could not be resolved using the commercial PMA BGE solution. Finally, the field amplified sample stacking (FASS) technique for online sample pre-concentration was used, resulting in an improvement in the sensitivity and detection limits (S/N = 3) by at least 3-fold. The optimized method was applied for the analysis of treated tailings pond samples.

## 2. Experimental

### 2.1 Chemicals

All chemicals used for this work were of analytical grade unless otherwise noted. PMA, HMOH (0.1 M), TMAOH (25% v/v in $H_2O$, trace select) and CTAB (> 99%) were all purchased from Sigma (Sigma-Aldrich, MO, USA). The pH of the BGE was adjusted to 7.0, 8.0.and 9.0 with triethanolamine (TEA; Sigma, MO, USA). CE grade sodium hydroxide solution (1 M) was purchased from Agilent (Agilent Technologies Canada Inc., Mississauga, Ontario). Stock solutions of sulfur oxyanions were prepared daily from sodium thiosulfate ($Na_2S_2O_3$, > 99.9% purity, Sigma, USA), sodium tetrathionate ($Na_2S_4O_6$, > 99.9% purity, Sigma, USA), sodium trithionate ($Na_2S_3O_6$), and potassium pentathionate ($K_2S_5O_6$). Sodium trithionate ($Na_2S_3O_6$), and potassium pentathionate ($K_2S_5O_6$) were synthesized and purified using modified known methods[25]. The description of the synthesis and characterization of the single crystals used in this work have been published elsewhere.[26] Nanopure water from Barnstead Nanopure II (Barnstead Nanopure, CA, USA) with ionic purity of 18.2 $M\Omega cm^{-1}$ was used for this work. All solutions were degassed and filtered with a 0.22 µm nylon syringe filter (Canadian Life Science, ON).

## 2.2 Instrumentation

All CE analyses were performed on an Agilent 7100 $^{3D}$CE System (Agilent Technologies Canada Inc., Mississauga, ON) equipped with a diode array UV-vis detector. Data was acquired at 350.10 nm with references at 191 nm, 200, 214 nm and 254.10 nm (to invert the negative peaks) and processed using Agilent OpenLAB Chromatography Data System (CDS) ChemStation Edition for corrected peak areas, peak widths and heights, and migration times. Bare fused-silica capillaries (internal diameter, 50 µm id) were obtained from MicroSolv Technology Corporation (NJ, USA) and were accurately cut to the desired length (48.5 cm). A 'window' was made 8.5 cm from detector by removal of the polyimide coating using the MicroSolv Window Maker™; approximately 2 mm of polyimide at the beginning and at the end of the capillaries was similarly removed. Initial conditioning was as follows: flushing at ~ 940 mbar with 1.0 M NaOH for 1 hour, water for 1 hour and BGE for another 1 hour. Daily the capillary was conditioned with 0.1 M NaOH for 10 min, water for 10 min and BGE for another 10 min. Between injections, the capillary was flushed with 0.1 M NaOH for 1 min, water for 1 min and BGE for 3 min. Except where stated otherwise, samples were injected hydrodynamically at 50 mbar for 10 s, and negative potential of between -20 kV and -30 kV was applied for the separation with indirect detection at the wavelengths specified above. The temperature of the capillary was maintained 25$^{o}$C for the separation. UV-vis analysis of the thiosalt species to determine their maximum absorption wavelengths ($\lambda_{max}$) and molar extinction coefficients were performed on a Varian Cary 6000i UV-Vis-NIR spectrophotometer

(Agilent Technologies Canada Inc., Mississauga, ON) with 1 cm quartz cuvettes (International Crystal Laboratories, NJ, USA).

### 2.3 Overview of factors studied

Table 2 shows the factors and the levels at which they were studied. Each factor was optimized in a univariate approach (one factor at a time).The rationale for choosing the selected ranges is based on previous experiments with thiosalt standards and analysis reported in literature.[6,9,16] For all factors, levels at lower and higher values than those reported are included to ensure that the optimum levels could be determined from this experiment without unnecessary constraints.

Table 2. Factors influencing separation and levels studied

| Factor | Levels |
|---|---|
| [PMA] (mM) | 1.00 – 1.50 – 2.00 – 2.50 – 3.00 (5 levels) |
| [TMAOH] (mM)<br>[CTAB] (mM)<br>[HM$^{2+}$] (mM) | 0.20 – 0.40 – 0.60 – 0.80 – 1.00 (5 levels) |
| pH | 7 – 8 – 9 (3 levels) |
| Applied potential (kV) | 20 – 25 – 30 (3 levels) |

## 3. Results and discussions

### 3.1 UV-vis analysis of thiosalts

UV-vis spectra were acquired for all the thiosalt species and BGE chromophoric probes to determine their molar absorptivities at the wavelengths of maximum absorbance ($\lambda_{max}$) to guide the choice of chromophoric probes for use for the experimental design. For the most sensitive results, analytes should not absorb at the $\lambda_{max}$ of the probe. The $\lambda_{max}$ for PMA was determined to be 214 nm (see Fig S1 in supporting document). Figure 1 shows the UV absorption spectra of the thiosalt species.

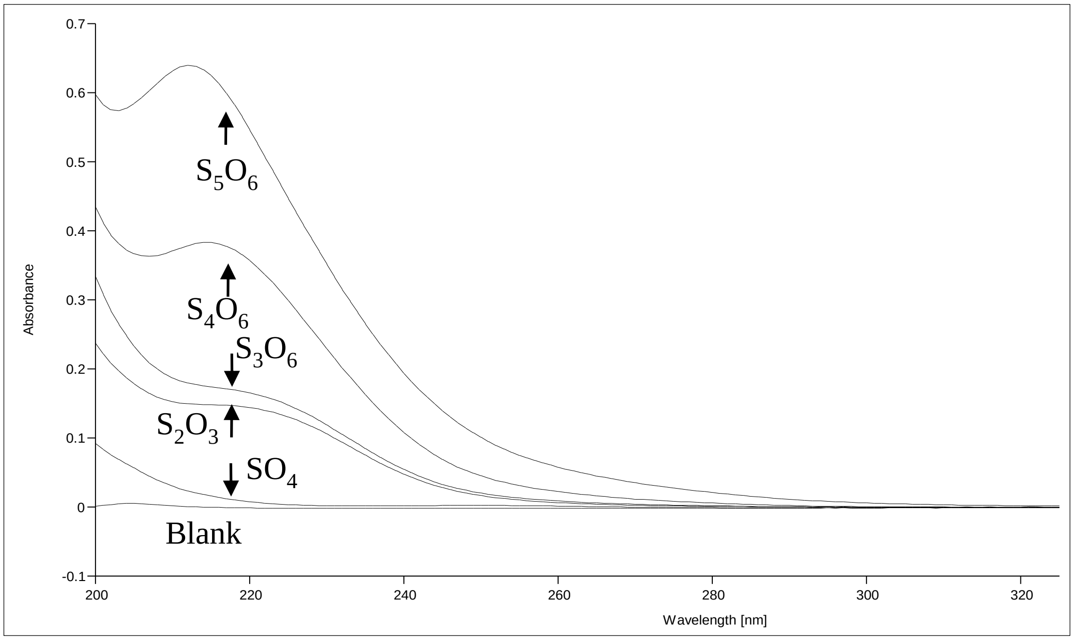
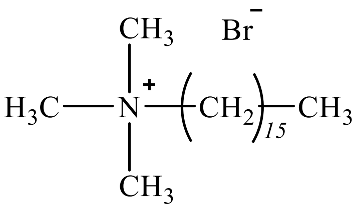


Figure 1: UV-vis absorption spectra of four thiosalt species (200 µg/mL each in water).

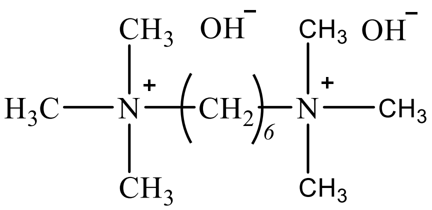
## 3.2 Influence of EOF modifier concentration

EOF modifiers are usually cationic surfactants that adsorb onto the surface of bare-fused silica due to strong electrostatic attraction between the negatively charged wall (Si-O$^-$) and the positively charged group of the modifier. This imparts a positive charged to the surface of the wall and greatly suppresses or reverses the EOF. In this work 3 EOF modifiers (Figure 2) were used: TMAOH, CTAB and HMOH. With a constant concentration of the PMA probe at 3.00 mM, [TMAOH], [CTAB] and [HMOH] were varied from 0.2 mM to 1.0 mM to test the ability to enhance the separation speed of the analysis. Since the electrophoretic mobilities of the sulfur anions are counter-EOF, suppressing or reversing the EOF will lead to rapid separation or resolution of the analytes.



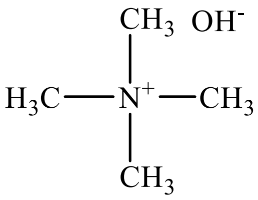
$$H_3C-N^{+}(CH_3)_2-(CH_2)_{15}-CH_3\ Br^-$$

Hexadecyltrimethylammonium bromide (CTAB)



$$H_3C-N^{+}(CH_3)_2-(CH_2)_6-N^{+}(CH_3)_2-CH_3\ OH^-\ OH^-$$

Hexamethonium hydroxide (HMOH)



$$H_3C-N^{+}(CH_3)_2-CH_3\ OH^-$$

Tetramethylammonium hydroxide (TMAOH)

Figure 2. EOF modifiers used in this work

As expected, the addition of all the EOF modifiers reduced the EOF flowing away from the detector, which led to higher effective electrophoretic mobilities of all the thiosalts species and faster migration towards the detector. Figure 3 shows the effective mobilities of the thiosalts species with respect to the concentration of the EOF modifiers. However, increases in [TMAOH] and [CTAB] even at low concentrations led to other undesirable separation issues.  As [TMAOH] was increased from 0.20 mM to 1.00 mM, total analysis time decreased from 10.7 min to 4.2 min, however under these conditions $SO_4^{2-}$ and $S_3O_6^{2-}$ were not resolvable. It was found that it was not possible to find a combination of conditions that would give short analysis time and satisfactory resolution using TMAOH, thus it was not used further. CTAB was also evaluated for its effectiveness and influence on the EOF modifier in many studies, for instance, Chen *et al.*[7] used CTAB with PMA in a TRIS buffer to separate $S_2O_3^{2-}$, $S_2O_3^{2-}$, $SCN^-$ and $SO_3^{2-}$ leading to reduced analysis time. For this work, CTAB led to a reduction of analysis time but it led to co-migration of $SO_4^{2-}$ and $S_3O_3^{2-}$ as well as caused broadening of the tetrathionate peak even at low concentrations (0.20 mM) and co-elution of the $SO_4^{2-}$ and $S_3O_6^{2-}$ peaks when increased further. HMOH was much more effective for regulating the EOF in the analysis of the thiosalt species than the other EOF modifiers, leading to good peak resolution, shorter analysis as well as much better peak symmetry for all the thiosalt species analyzed. Thus HMOH was used for further optimization of the BGE for the analysis. The optimum concentration of [HMOH] was found to be 0.80 mM giving full separation in 4.4 min; above this concentration, $S_2O_3^{2-}$ and $S_5O_6^{2-}$ start to co-migrate and the $S_4O_6^{2-}$ peak begins to broaden. Thus [HMOH] = 0.80 mM was used for further optimization.

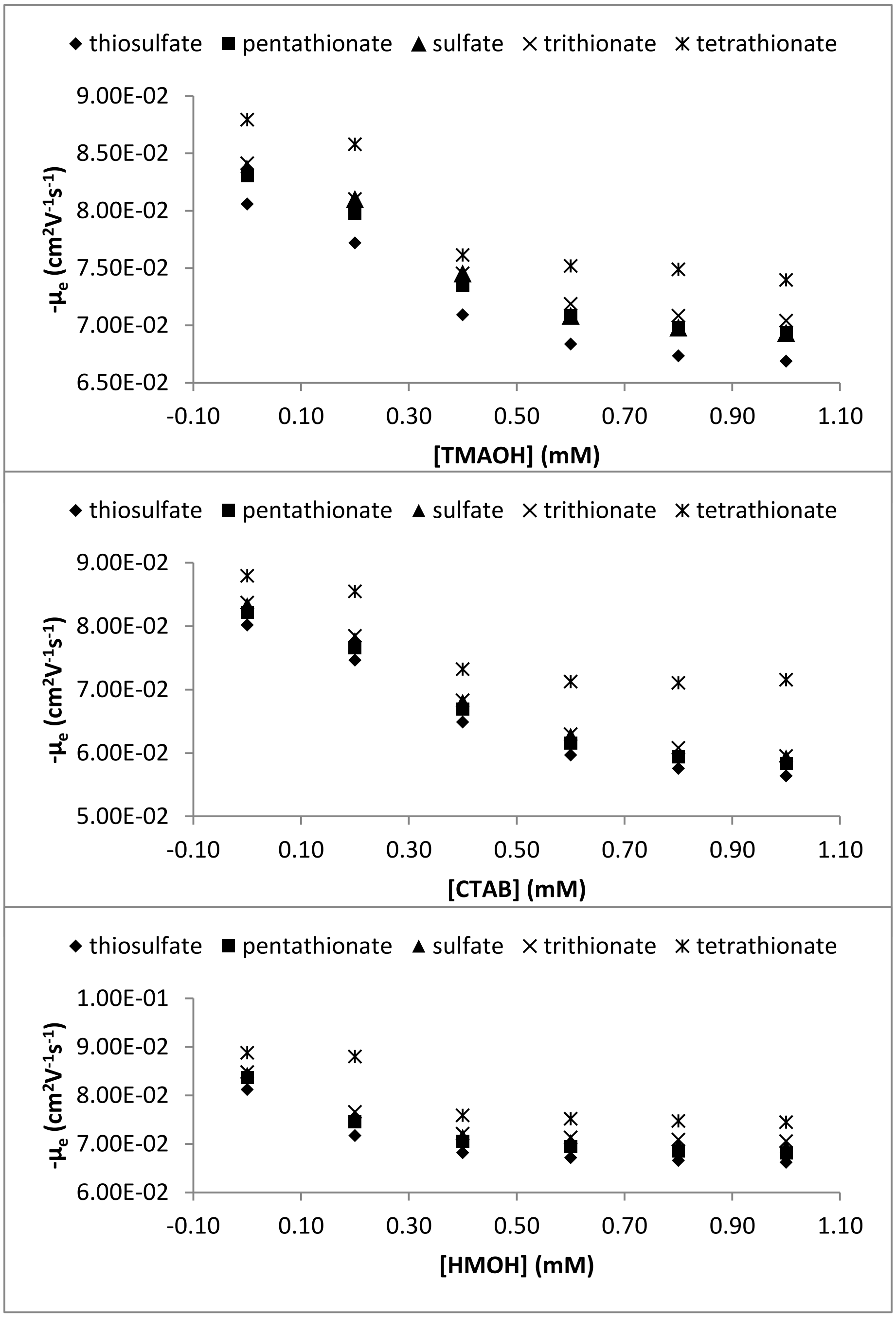


Figure 3. Effect of increasing [TMAOH], [CTAB] and HMOH] on effective electrophoretic mobilities of thiosalt species

### 3.3 Influence of PMA chromophoric probe concentration

PMA was chosen for this work because of its high molar absorptivity and close mobility match with those of the sulfur oxy-anions leading to improved sensitivity and reduced electromigration dispersion; has no oxidizing properties or reactivity with thiosalts anions and has a high molar extinction coefficient at the chosen wavelength for this work.[7,18,19] With the optimal concentration of HMOH at 0.80 mM, the concentration of the PMA was varied from 1.00 mM to 3.00 mM with 5 concentrations in total (see Fig. S5 in supporting document). With PMA at 1.00 mM, the peak symmetry and sensitivity (with respect to peak height, width and area) were very poor for all the thiosalt species; at 2.00 mM PMA gave higher sensitivity and good peak symmetry with faster migration (last peak at 4.3 min) than at all the other concentrations. Further increase in the concentration of PMA beyond 3.00 mM led to a slight increase in signal sensitivity, but poor peak symmetry (tailing and fronting), indicating that there may be some interaction of the BGE probe with the anions.

### 3.4 Influence of pH and applied field on the electrophoretic mobilities of thiosalts

The use of the EOF modifier should in principle either slow down or reverse the EOF and therefore lead to higher migration of the thiosalts anions towards the detector. An increase in pH will lead to increase in the ionization of the silanol group on the surface of the capillary. The increase in positive charge on the surface of the capillary by the addition of cationic EOF modifiers such as HMOH will therefore increase with increasing pH until all the available silanol groups are ionized. With the BGE at pH 7 with [PMA] = 2.00 mM and [HMOH] = 0.80 mM, the migration times of the thiosalt species ranged from 3.8 min to 5.7 min (see Figure S6); at pH 8 migration times reduced to a range of 3.1 min to 4.3 min; and at pH 9 the range was 3.4 min to 4.9 min. At pH 9, migration times were slightly longer than at pH 8, and $S_2O_3^{2-}$ and $S_5O_6^{2-}$ co-migrated. With the optimum BGE composition of [PMA] = 2.00 mM, [HMOH] = 0.80 mM and BGE pH of 8, the applied separation field was varied from -20 kV to -30 kV (see Figure S7). As expected, with increased potential the time for separation was reduced from 4.2 min (-20 kV) to 2.8 min (-30 kV).

### 3.5 Optimization of sensitivity

Detection wavelengths for most capillary electrophoretic analysis with indirect detection of sulfur anions have been performed at $\lambda = 254$ nm. The UV-vis diode array detector (DAD) allows for monitoring at different wavelengths, which allows for selection of wavelengths that give the most sensitivity. Wavelengths selected were 191 nm, 214 nm, 200 nm and 254 nm. Although most of these are not at the $\lambda_{max}$ for the PMA chromophoric probe (214 nm), the absorbance of the UV active thiosalts influence the sensitivity (usually decreasing sensitivity for indirect mode). The most sensitive results for sulfate, which has low absorptivity (Figure 1), should be seen at 214 nm. However, for the more UV active species, 200 nm on average gave the best sensitivity and was therefore used for further analysis. To invert the negative peaks, measurements were taken at 350 nm and 200 nm was used as the reference wavelength; since the decrease in absorbance at 200 nm will be greater than that at 350 nm, this will register as a positive peak. The optimized buffer system was determined to be: 2.00 mM PMA, 0.80 mM $HM^{2+}$, pH adjusted to 8.0 with triethanolamine. All five sulfur oxyanions species under investigation were successfully separated (Figure 4) under these conditions in less than 3 min. To the best of our knowledge, this is the fastest CZE method with indirect UV-vis analysis reported so to date for all these species.

### 3.6 Linearity of method, sensitivity and LOD determination

Calibration curves were constructed for all thiosalts species at six concentrations (including a blank). Each standard sample was injected in triplicate and the standard deviation of the peak areas and migration times determined. Very good linearity with $R^2 > 0.99$ was obtained from the calibration curves for all the thiosalts species (Figure 4). LOD values were obtained at S/N ratios of 3 by sequential dilution of thiosalt mixtures until S/N value of the peak of interest reach 3. Table 3 shows the relative standard deviations (RSD) of the migration times and peak areas, linearity of standard calibration curves and LOD values for each of the thiosalt anions.

Field-amplified sample stacking (FASS) was applied to increase the sensitivity of the method and to achieve low LOD values. In this work, FASS was applied by injecting a small plug of water (1 – 5 s at 30 mbar) into a BGE-filled capillary, injecting the sample plug (5 s at 50 mbar) and applying high negative potential. LOD values for stacking and normal CZE are shown in Table 3 leading to a nearly 4-fold increase over normal hydrodynamic injection for some of the thiosalts (see Figure S10 in supporting documents). No further increase in sensitivity was observed after 3 s injection time of water plug before the sample. Larger volumes of water (more than 5 s at 30 mbar) led to decrease in sensitivity probably due to sample dilution.

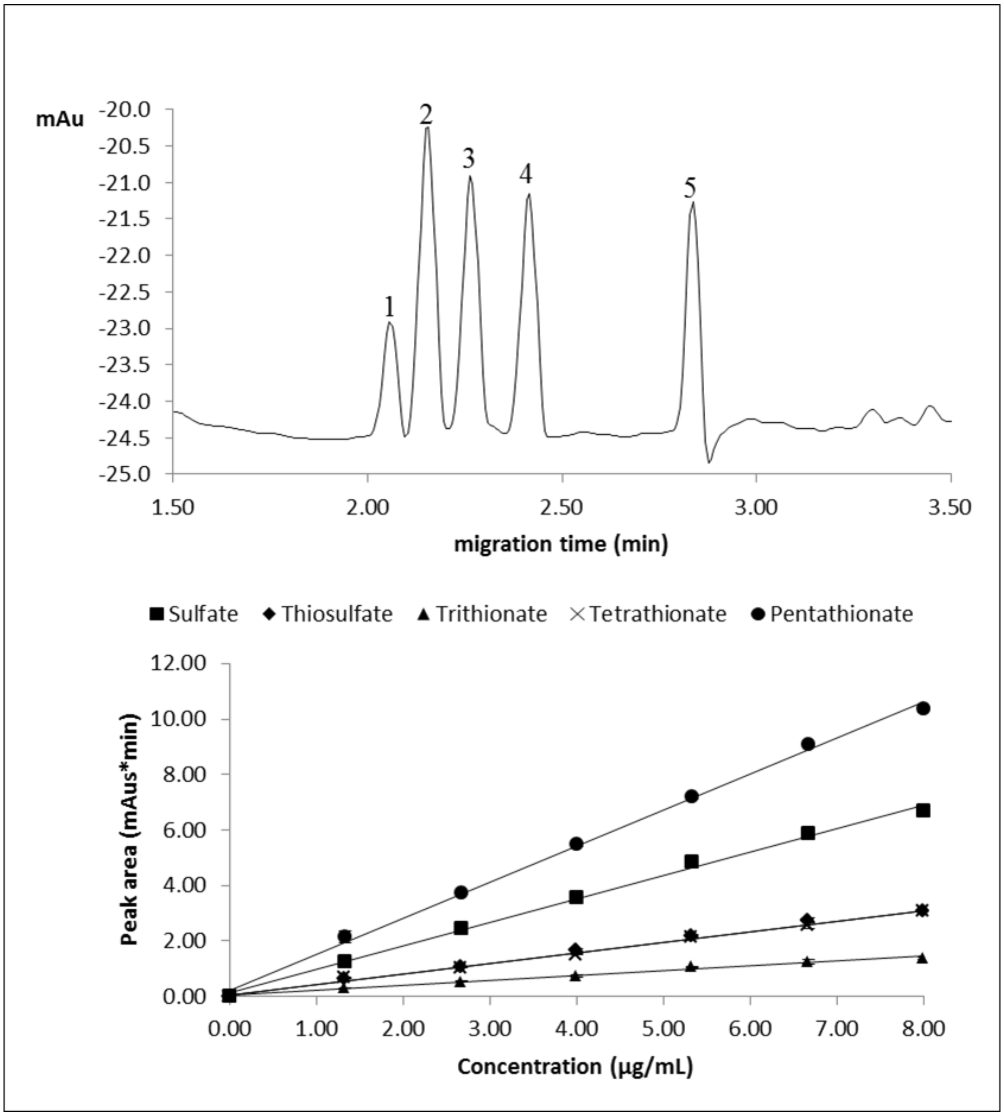


Figure 4: Electropherogram and standard calibration curves obtained from thiosalt standards. 1. $S_2O_3^{2-}$ (40 µg/mL), 2. $S_5O_6^{2-}$ (40 µg/mL), 3. $SO_4^{2-}$ (20 µg/mL), 4. $S_3O_6^{2-}$ (50 µg/mL), $S_4O_6^{2-}$ (50 µg/mL). CZE conditions: injection: 250 mbar.s, applied field: -30 kV, temperature: 25 ºC, indirect UV detection at $\lambda$ = 350 nm, $\lambda_{ref}$ = 200 nm. BGE 2.00 mM PMA, 0.80 mM $HM^{2+}$, pH adjusted to 8 with TEA

312 Table 3. Peak area and migration time repeatability, linearity of standard calibration and

313 LOD values of thiosalt anions analysis

| Thiosalt anion | Concentration (μg/mL) | RSD peak area (%) (n=3) | RSD migration time (%) (n=3) | coefficient of regression ($R^2$) | LOD without stacking (n=3) (μg/mL) | LOD with stacking (n=3) (μg/mL) |
|---|---|---|---|---|---|---|
| $SO_4^{2-}$ | 1 | 0.50 | 0.06 | 0.9953 | 0.09 | 0.02 |
| | 5 | 0.51 | 0.14 | | | |
| | 10 | 0.58 | 0.06 | | | |
| | 15 | 0.87 | 0.04 | | | |
| | 20 | 0.53 | 0.14 | | | |
| $S_2O_3^{2-}$ | 1 | 2.95 | 0.08 | 0.9998 | 0.16 | 0.12 |
| | 5 | 2.70 | 0.10 | | | |
| | 10 | 1.55 | 0.06 | | | |
| | 15 | 0.53 | 0.02 | | | |
| | 20 | 1.28 | 0.17 | | | |
| $S_3O_6^{2-}$ | 1 | 0.85 | 0.09 | 0.9454 | 0.34 | 0.11 |
| | 5 | 1.46 | 0.14 | | | |
| | 10 | 3.93 | 0.12 | | | |
| | 15 | 2.42 | 0.12 | | | |
| | 20 | 3.31 | 0.05 | | | |
| $S_4O_6^{2-}$ | 1 | 1.40 | 0.02 | 0.9940 | 0.32 | 0.14 |
| | 5 | 3.77 | 0.14 | | | |
| | 10 | 2.85 | 0.06 | | | |
| | 15 | 1.74 | 1.68 | | | |
| | 20 | 0.39 | 0.03 | | | |
| $S_5O_6^{2-}$ | 1 | 0.47 | 0.70 | 0.9952 | 0.10 | 0.04 |
| | 5 | 0.54 | 0.10 | | | |
| | 10 | 0.54 | 0.06 | | | |
| | 15 | 0.92 | 0.04 | | | |
| | 20 | 0.53 | 0.13 | | | |

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### 3.7 Comparison to commercially available PMA BGE for inorganic anions

A PMA BGE is commercially available Agilent Technologies, ON) for the analysis of inorganic anions, but is costly at least 60 times more than the reagents used in the BGE reported here. The PMA BGE developed for this study was compared to the commercially available BGE and was found to be more sensitive (greater peak areas in optimized PMA BGE) with better separation efficiency (Figure 5) under all injection and applied field conditions.

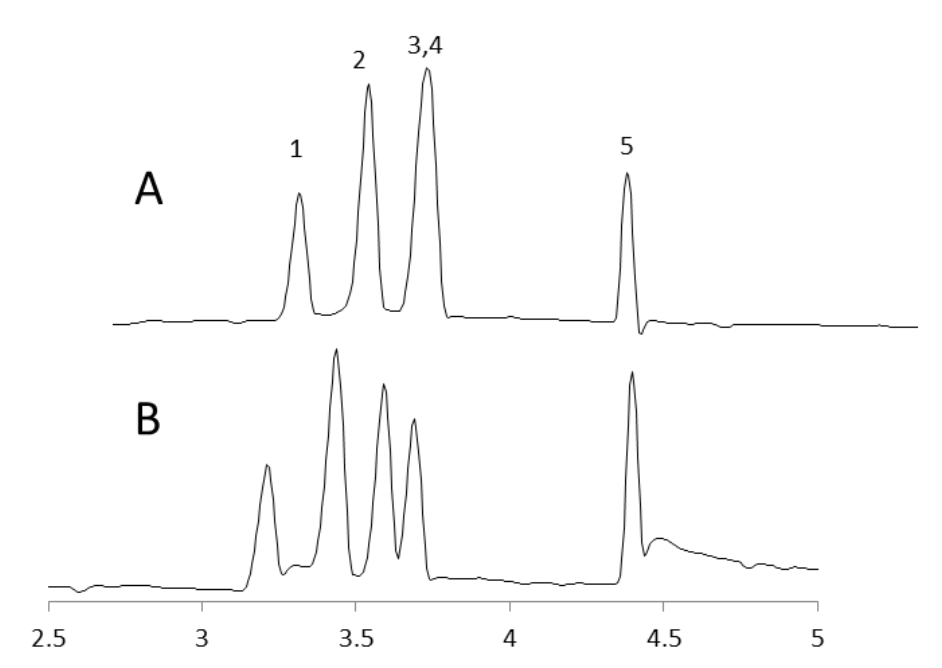

Figure 5. Electropherogram showing separation efficiency of five thiosalts species using commercially available PMA BGE (A) with the optimized PMA BGE (B). 1. $S_2O_3^{2-}$ (40 µg/mL), 2. $S_5O_6^{2-}$ (27 µg/mL), 3. $SO_4^{2-}$ (23 µg/mL), 4. $S_3O_6^{2-}$ (50 µg/mL), 5. $S_4O_6^{2-}$ (50 µg/mL). Separation conditions: hydrodynamic injection, 300 mbar.s; applied field: -20 kV

### 3.8 Application of method to thiosalt standard mixture and tailings pond samples

The final BGE composition and instrument parameters were applied to the analysis of thiosalt standards and samples taken from a thiosalt tailings pond. The method was rapid with analysis time of less than 3 min, sensitive, gave good resolution of all peaks and proved reliable for the thiosalt species analyzed. Optimal detection was achieved at 350 nm against a reference wavelength of 200 nm with good peak intensity, reproducibility and linearity for all the anions under consideration over the concentration range of 1 µg/mL to 20 µg/mL. Electropherograms of diluted spiked tailings pond water (1:100 with nano-pure water) and diluted spiked tailings pond water along with the standard addition plots of the five thiosalts are presented in Figure 6. Based on this dilution, the total method detection limits would be 10 and 30 µg/mL for the highly complex tailings waters. It should be noted that typical thiosalts concentrations are well above 50 µg/mL, and at these concentrations are considered of low toxicity to fish so these detection limits are adequate. By sacrificing short analysis times sought for this work, it is possible to analyze pond samples with little or no dilution and still accommodate the relatively high concentrations of sulfate and thiosulfate. For less complex samples, as measured in kinetic and thermodynamic studies, dilution is not necessary and the high method sensitivity is maintained.  To prepare the samples for standard addition, 100 µL of the 1:100 diluted sample was spiked with 0, 100, 200, 300, 400 and 500 µL of the thiosalt standard mix (0.33 µg/mL sulfate, 0.45 µg/mL thiosulfate and 0.88 µg/mL each trithionate, tetrathionate and pentathionate), made up to 600 µL, mixed with a vortex and analyzed. Three samples were prepared for each standard addition point to determine the standard deviations associated with each measurement. Table 4 shows the results of the standard addition analysis. The main thiosalts present detected in the sample taken from the tailings pond were sulfate ($SO_4^{2-}$) and thiosulfate ($S_2O_3^{2-}$) with concentrations about 300 μg/mL for $SO_4^{2-}$ and 700 μg/mL for $S_2O_3^{2-}$. The question of the necessity of dilution and application of standard addition to address matrix effects was evaluated by comparing ratios of the slopes of standard addition and standard calibration plots for each thiosalt species. The ratios ranged from 1.4 to 3, with higher slopes seen in the analysis by standard addition. The effect was not consistent, which shows that matrix components influence the analysis and confirms necessity of using a standard addition method.

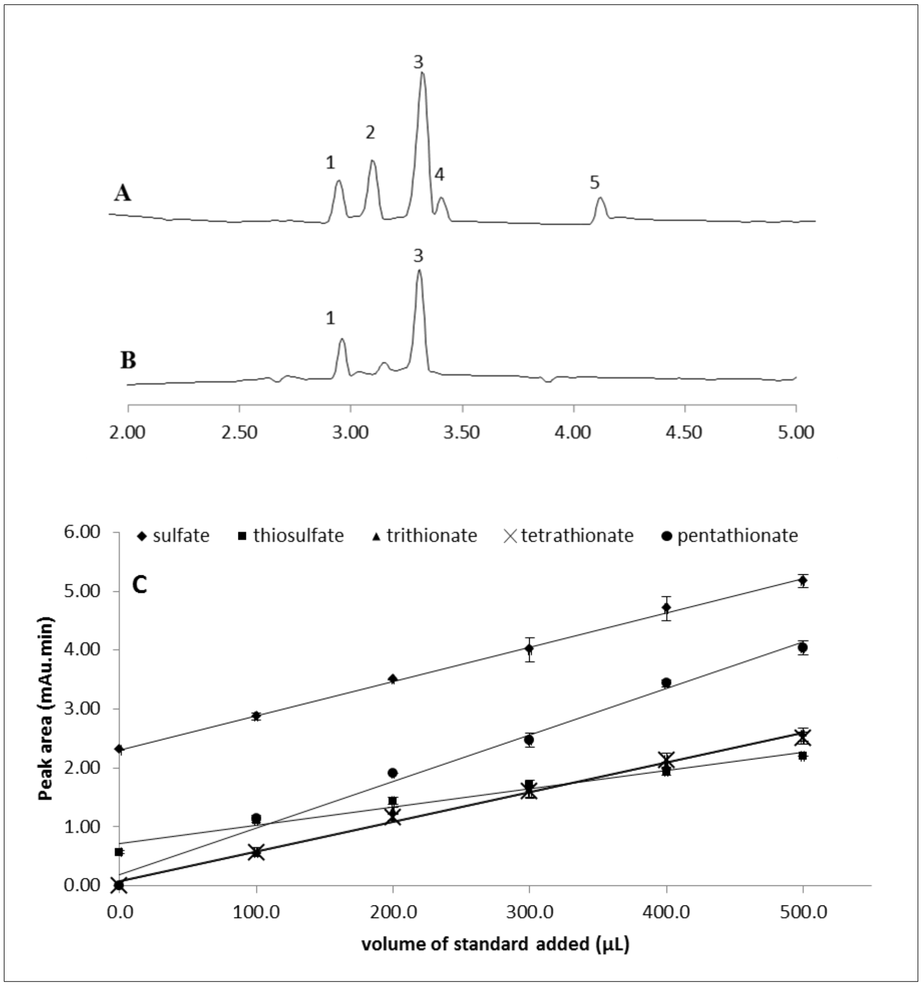

Figure 6: Electropherograms of A. Real sample spiked with 0.28 µg/mL sulfate, 0.38 µg/mL thiosulfate, and 0.73 µg/mL trithionate, tetrathionate and pentathionate. Peaks: 1. $S_2O_3^{2-}$, 2. $S_5O_6^{2-}$, 3. $SO_4^{2-}$, 4. $S_3O_6^{2-}$, 5. $S_4O_6^{2-}$; B. Real thiosalt tailings sample diluted 1:100. C. Standard addition calibration curves of thiosalts. CE conditions: injection: 250 mbar.s, applied field: -20 kV, temperature 25 °C, BGE: 2.00 mM PMA, 0.80 mM $HM^{2+}$, pH adjusted to 8.0 with TEA and indirect UV detection at $\lambda = 350$ nm, $\lambda_{ref} = 200$ nm

Table 4. Standard addition result of thiosalt sample spiked with 0, 100, 200, 300, 400 and 500 µL thiosalt standard mix (n=3)

| Thiosalt anion | Average migration time (min) | Concentration determined (µg/mL±SD) |
|---|---|---|
| $SO_4^{2-}$ | 2.39 | 324.3 ± 31.1 |
| $S_2O_3^{2-}$ | 2.14 | 709 ± 74.1 |
| $S_3O_6^{2-}$ | 2.47 | 43.5 ± 19.6 |
| $S_4O_6^{2-}$ | 2.92 | 9.4 ± 7.8 |
| $S_5O_6^{2-}$ | 2.30 | 6.1 ± 5.5 |

The high standard deviation values for the higher order polythionates was probably due to their very low concentrations (below their detection limit) or absence from the tailings sample.

## 4. Conclusions and future work

A fast CE method with indirect UV-vis detection was developed for the separation of five important thiosalts anions: sulfate ($SO_4^{2-}$), thiosulfate ($S_2O_3^{2-}$), trithionate ($S_3O_6^{2-}$), tetrathionate ($S_4O_6^{2-}$) and pentathionate ($S_5O_6^{2-}$). The optimized BGE consisted of 2.00 mM PMA, 0.80 mM $HM^{2+}$, pH adjusted to 8.0 with triethanolamine and indirect UV detection at $\lambda$=200 nm. The method was rapid, sensitive, selective and reliable with complete separation of the thiosalt species under 3 min. LOD values (from 0.1 µg/mL to

0.34 µg/mL) were improved by about 4 fold (to 0.02 to 0.12 µg/mL) with the use of field-amplified sample stacking (FASS), first injection of a plug of water for 3 s at 30 mbar followed by sample injection for 5 s at 50 mbar. Results from the experiments showed that HM as EOF modifier was superior to CTAB and TMAOH for thiosalt analysis. The optimized method was successfully applied to the quantitative analysis of thiosalts species in tailings pond samples with challenges posed by the complexity of the tailings matrix accommodated.

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