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Journal of Chromatography A, 745 (1996) 111–115

JOURNAL OF CHROMATOGRAPHY A

Simultaneous analysis of selenate and selenite in bacterial suspensions by capillary electrophoresis

Edward B. Walker*, Jeffrey C. Walker, Spencer E. Zaugg, Robert Davidson

Center for Chemical Technology, Weber State University, 2503 University Circle, Ogden UT 84408-2503, USA

Abstract

The use of capillary electrophoresis allows simultaneous determination of selenate and selenite during reduction by whole bacteria or their lysates. Optimal separation was achieved with 20 m*M* sodium borate and 1.0 m*M* tetradecyltrimethylammonium bromide, pH 9.5, −25 kV, at 28°C in an open, uncoated 44 cm×75 μm silica column. This method eliminates many steps in sample preparation and reduces necessary sample size. Application of this CE method in studying the depletion of both selenate and selenite in bacterial suspensions is reported.

Keywords: Selenate; Selenite

1. Introduction

Selenium constitutes approximately 0.09 ppm of the earth's crust. It is an essential trace element in animal diets at levels of approximately 0.1 ppm, but is toxic at higher concentrations [1]. Selenium is a dark red-colored solid in its elemental form, but also exists in two other valences of four and six, the most common of which are selenite and selenate, respectively. Selenium is used in manufacturing products such as photocells, semiconductors, and glass pigments. It is also present in refining tailings, sewage sludge, agricultural drainage, and exhaust gases from burning fossil fuels [2–5]. As a result, selenium pollution represents a major concern in some areas, with selenate and selenite being the most common forms of selenium in polluted water.

Bioremediation using various microorganisms capable of reducing selenate or selenite into elemental selenium has gained attention in studies that have been conducted over the last 30 years [6–9]. Often, the elemental selenium is retained by bacteria, giving them a characteristic red color. We report the application of capillary electrophoresis in the simultaneous analysis of selenate and selenite in a complex bacterial growth medium.

Most traditional analytical methods for selenium involve its conversion into either selenate or selenite and subsequent titration [9], fluorescence spectroscopy [10], or atomic absorption (AAS) [11]. Sodium also interferes with selenium determination by AAS, thus necessitating separation of selenium from sodium during sample preparation [12]. The use of ion chromatography has helped in the analysis of selenate and selenite. The use of HPLC to analyze various inorganic and organic forms of selenium is reviewed by Koelbl et al. [13]. Coupling HPLC to ICP-MS enhances the usefulness of this technique in studying the metabolic pathways of selenium [14].

Earlier papers by other investigators have dealt with the simultaneous determinations of selenium and arsenic oxoanions [15–17]. Vogt and Werner reported a separation method for selenate and selenite from arsenic oxoanions and heavy metal com-

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00378-0

plexes using indirect detection [15]. Yoshida and Hida studied additives to enhance separation of selenate and selenite from tellurate and tellurite by means of capillary electrophoresis [18]. However, these previously-reported methods were not sufficient to resolve selenate and selenite in the highly complex solutions of bacterial growth media used in our studies of bioremediation. We have investigated the use of capillary electrophoresis (CE) to study selenate and selenite reduction in bacterial suspensions. We report the application of CE in quantitating the depletion of both these ions by bacteria in complex growth media.

2. Experimental

2.1. Apparatus

Separations were performed using a Spectrophoresis 1000 CE system, containing an autosampler, capillary temperature control, and a rapid-scan UV–Vis detector (ThermoSeparations Products, San Diego, CA, USA). The instrument was interfaced to a Pentium 90 computer running PC1000 ThermoSeparation Products software, Version 3.0.

Uncoated, open, 75 μm diameter silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA) and cut to 44 cm.

2.2. Reagents

Reagents were obtained from the following sources: Sodium borate, decahydrate from EM Sciences (Gibbstown, NJ, USA), tetradecyltrimethylammonium bromide (TTAB) from Sigma (St. Louis, MO, USA), sodium selenite and sodium selenate from Aldrich (Milwaukee, WI, USA), and tryptic soy broth (TSB) from Difco (Detroit, MI, USA). Water was distilled and deionized before use.

2.3. Bacteria

An isolate of *Pseudomonas stutzeri* was obtained from the US Bureau of Mines (Research Center, Salt Lake City, UT, USA) which was used throughout the study. Organisms were grown in TSB medium (pH 7.2) containing 100 ppm sodium selenate at 37°C under aerobic conditions for 48 h prior to the beginning of the experiment. Final concentration of bacteria during the experiments was 10^8/ml.

2.4. Electrophoresis conditions

Silica capillaries were initially conditioned with 1 *M* NaOH for 5 min at 60°C, then with 0.1 *M* NaOH for 5 min at 60°C, followed by water at 28°C. The capillary was washed with two volumes of fresh running buffer prior to each injection. The running buffer consisted of 20 m*M* sodium borate and 1.0 m*M* TTAB at pH 9.5. The sample was injected using electrokinetic injection at −18 kV for 1 s or by hydrodynamic (vacuum) injection for 2 s. Separations were run at −25 kV and 28°C. Rapid scanning was used to collect UV spectra from 192 to 300 nm during each separation. Electropherograms were generated for selenate and selenite by extracting 200 nm absorption data during post-run processing.

2.5. Procedure

Samples of the bacteria–selenium suspensions were taken from culture flasks, placed into CE injection vials daily, and frozen until time of analysis. All samples were thawed and analyzed as a single batch. No other sample preparation was necessary.

3. Results

3.1. Electropherograms

As may be seen from Figs. 1 and 2 the peaks for selenate and selenite are well-resolved from each other and from other substances in the complex TSB medium. Both peaks elute in approximately 5 min (2.5 min for selenate and approx. 5 min for selenite). Changes in concentration of these ions are readily observed over a period of 3 days.

3.2. Calibration and sensitivity

Excellent resolution, sensitivity, and linear standard curves were achieved utilizing 1 s electrokinetic injections at −18 kV. Calibration curves for selenate

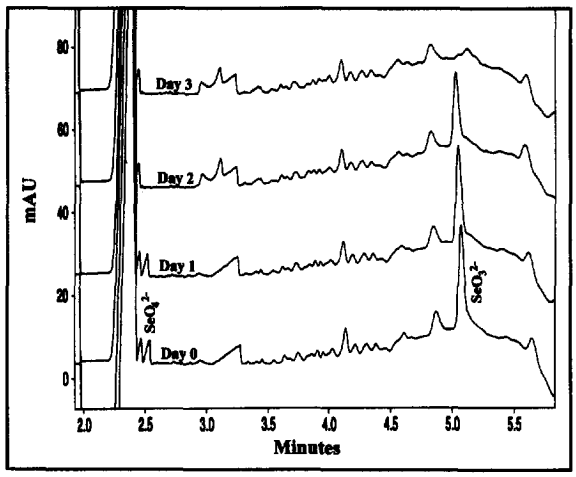

Fig. 1. Electropherograms of daily samples taken from a bacterial suspension in TSB at 20°C. The running buffer consisted of 20 m*M* sodium borate and 1.0 m*M* TTAB at pH 9.5; electrokinetic injection at −18 kV for 1 s; separation at −25 kV and 28°C; detection at 200 nm.

and selenite were linear, with selenite yielding a much stronger detector response (five points; selenate: slope 52.2, intercept 14.4, std. err. of coef. 0.732, r^2=0.9998; selenite: slope=96.2, intercept −11.8, std. err. of coef. 0.819, r^2=0.9999). Electrokinetic injections yielded 100 times more sensitivity

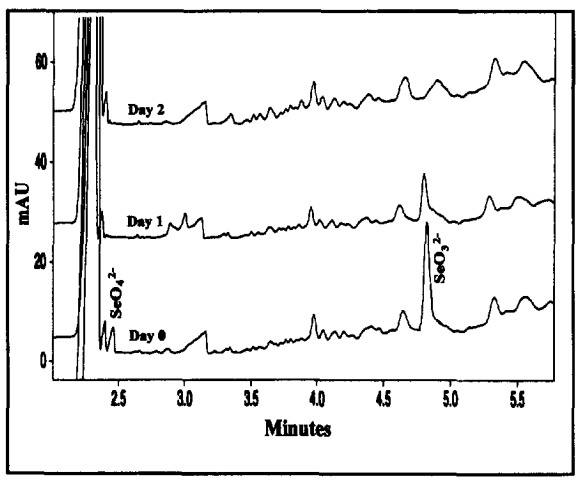

Fig. 2. Electropherograms of daily samples taken from a bacterial suspension in TSB at 37°C. The running buffer consisted of 20 m*M* sodium borate and 1.0 m*M* TTAB at pH 9.5; electrokinetic injection at −18 kV for 1 s; separation at −25 kV and 28°C; detection at 200 nm.

than hydrodynamic injections for both ions. Observed limits of detection for selenate and selenite sampled directly from bacterial growth medium were 2 and 0.4 ppm respectively.

3.3. Selenate and selenite reduction

Selenate and selenite are reduced by *Pseudomonas stutzeri* at different rates over a period of 3 days as seen in Figs. 1 and 2. Reduction of both selenium ions is faster at 37°C compared to 20°C. However, at both temperatures, selenate is reduced first, followed by selenite (summarized in Fig. 3). Within the first hour of mixing bacteria in media containing 230 ppm selenate, half of the selenate is reduced to selenite. The remaining selenate is reduced at a much slower rate, taking one or two days at 20°C or 37°C respectively. The selenite formed from the reduction of selenate is subsequently reduced to elemental selenium. However, this reaction proceeds more slowly than the initial selenate reduction.

4. Discussion

CE offers an excellent method for simultaneous rapid analysis of selenate and selenite in complex aqueous solutions and bacterial suspensions with little or no sample preparation. The ability to sample directly from a complex suspension of bacteria and TSB media underscores the high resolution separation power of CE. The electrophoretic mobility of the relatively small selenate and selenite ions, coupled with their UV absorption at 200 nm make these ions excellent candidates for CE analysis. Selenate and selenite migrate ahead of many potentially-interfering substances. Extended CE run times resolve hundreds of compounds at retention times of 6–30 min (data not shown).

The key to successful resolution of the selenate from a neighboring peak is adjustment of the pH to 9.5 and a temperature of 28°C. Raising the pH above 9.7 or lowering it below 9.2 resulted in loss of resolution. Higher temperatures moved the selenate into the EOF frontal detector noise and lower temperatures retarded selenite's migration time, causing interferences from later-migrating ions. Other buffers such as glycinate and phosphate at the

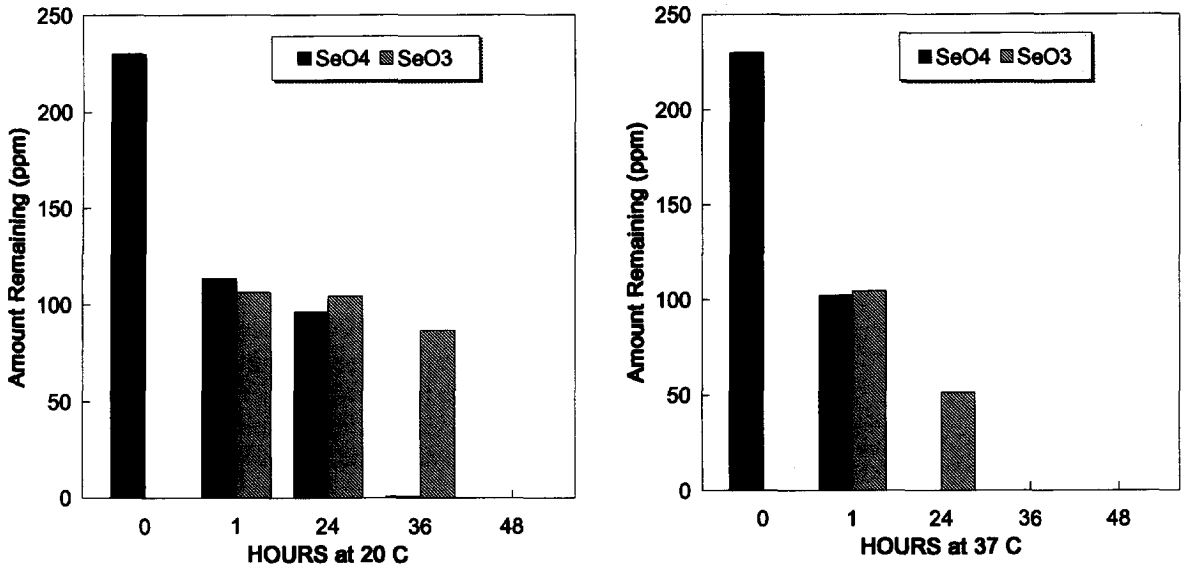

Fig. 3. Changes in concentrations of selenate and selenium in bacterial suspensions over a period of 3 days at 20°C and 37°C.

same pH values did not allow resolution of selenate. A very small peak (2.4 min) that remains after selenate is removed from solution (Figs. 1 and 2), was not selenate and did not interfere with the concentration determinations.

Detection limits of 2 ppm for selenate and 0.4 ppm of selenite are a result of both the sensitivity of the UV detector and injection method. The higher sensitivity toward selenite over selenate was also seen in the earlier, independent electropherograms of Yoshida and Hida [15]. Hydrodynamic injections of up to 15 s yielded only small peaks for either selenate or selenite. On the other hand, electrokinetic injections of 1 s at −15 to −20 kV yielded excellent results for both ions. Electrokinetic injection times longer that 2 s or higher voltages yielded non-linear calibration curves, especially at selenate concentrations above 50 ppm. Longer electrokinetic injection times at higher voltages offer proportionally lower detection limits, yet we found that interfering substances from the bacterial medium were not easily resolved from selenate. Sampling selenate from deionized water solutions, using 20 s electrokinetic injections at −30 kV, allowed detection levels of selenate at 0.6 ppm and selenite at 0.2 ppm (S/N ratio>3).

Both selenate and selenite are reduced by *Pseudomonas stutzeri*, which confirms the observations of earlier investigators [12]. The initial oxidation of selenate to selenite occurs much more rapidly than was anticipated. Approximately one-half of the selenate is converted to selenite within 1 h of mixing with the bacteria. During the next 24 h, the oxidation of selenate slows dramatically. The reduction of selenite occurs more slowly and appears to be a rate-limiting step in the conversion of selenate to elemental selenium via selenite. The reasons for this may include the availability of reductant(s) which we have not yet identified. Alternatively, it may be a result of bacteria preferentially absorbing selenite. The CE method for analysis selectively samples from the growth medium in whole-cell suspensions of bacteria, not the internal contents of the bacteria. Our data demonstrates that the selenate-to-selenite conversion occurs externally, allowing the observation of both these ions via CE. Microscopic observations of the bacteria, reveals that the red, elemental selenium is contained inside the bacterial cells. This suggests that selenite is may be absorbed into the bacteria and that the final reduction of selenite to selenium occurs inside the cells. Our work is continuing to identify the sources of electrons involved in selenate and selenite reduction and to study the locations and mechanism of this process.

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