Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Ketterer, Michael E. , Kozerski, Gary E. , Carroll, John , Ritacco, Robert and Painuly, Prabha(1997) 'Investigation of Capillary Free-Flow Electrophoresis for Separation of Co, Cr, and As Species in Aqueous Solution', Separation Science and Technology, 32: 1, 641 — 654
To link to this Article: DOI: 10.1080/01496399708003220
URL: http://dx.doi.org/10.1080/01496399708003220

SEPARATION SCIENCE AND TECHNOLOGY, 32(1–4), pp. 641–654, 1997

INVESTIGATION OF CAPILLARY FREE-FLOW ELECTROPHORESIS FOR SEPARATION OF Co, Cr, AND As SPECIES IN AQUEOUS SOLUTION

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ABSTRACT

Application of a prototype capillary free-flow electrophoresis (CFFE) device for separation of different solution species of Co, Cr, and As is explored. A unique free-flow electrophoresis (FFE) design is employed, which makes use of internal capillary cooling tubes to minimize thermal convection due to Joule heating.

INTRODUCTION

The development of sensitive plasma spectrochemical methods of elemental analysis represents a significant benchmark in the field of analytical chemistry. However, these techniques provide no information as to the chemical form of the element of interest as it existed in the sample, *prior* to introduction into the plasma. Additionally, the nature of the sample may dictate the need for analyte preconcentration or the removal of sample matrices which may interfere with the analysis. It is therefore apparent that chemical separations must play a role in the analysis if such information is desired. The uses of solvent extraction, ion exchange, high performance liquid chromatography (HPLC), and capillary electrophoresis (CE) have proven to be important tools for performing *batch* mode chemical speciations prior to spectrochemical analysis.

Capillary free-flow electrophoresis (CFFE), as it is described herein, is a relatively new separation tool having a significant number of advantages over the techniques listed above. The primary advantage of CFFE is that it is a continuous separation technique . Once a separation is begun, it will continue unattended for an indefinite period, given an adequate supply of buffer solutions and sample. This is useful to the analytical chemist in that a continuous analyte signal can be monitored, instead of the type of transient signal associated with capillary electrophoresis (CE) or HPLC. This is especially important in elemental mass spectrometry, where precision is governed by the cumulative number of detector events observed, i.e. counting statistics. In addition, CFFE separations are simple to perform and do not make use of a supporting medium, thereby avoiding potential deleterious effects of analyte-stationary phase interaction. CFFE makes use of dilute aqueous buffer/electrolyte solutions, typically a few millimolar, which are highly compatible with plasma spectrochemical techniques, as well as other common detection methods such as UV absorbance and conductivity. This work is undertaken to evaluate the feasibility of developing analytical speciation methods and/or semi-preparative scale separations of small molecules based upon CFFE. Applications of CFFE to date have focused primarily on biomolecules. These larger molecules undoubtedly have much smaller diffusion coefficients than those of the inorganic species in this work. Consequently, resolution of small analytes may be expected to be a non-trivial endeavor.

The results of speciation of substitutionally inert Co(III) complexes, $[Co^{III}(CN)_6]^{3-}$ and $[Co^{III}$ (sepulchrate)$]^{3+}$ ions, are reported. Additionally, CFFE has been successfully applied to aqueous solutions containing anionic Cr(VI) and aquo Cr(III) ions, with complete resolution of $[Cr^{III}(H_2O)_6]^{3+}$ and $Cr^{VI}O_4^{2-}$ species. Partial separation of common solution forms of As, namely arsenate, arsenite, and dimethylarsinic acid, is achieved with judicious selection of carrier buffer pH.

Capillary Free-Flow Electrophoresis

Free-flow electrophoresis (FFE), as first introduced around 1960 by Barrolier et al.(1) and Hannig(2), has been utilized as a continuous separation technique of biomolecules for a number of years. Interest in FFE as a preparative-scale separation technique in biotechnology has been generated recently. A vast array of potential applications of FFE to separations of inorganic species and coordination compounds exist which have hitherto not been explored.

Recent developments in scaling up FFE have been demonstrated(3,4), which overcome the problem of thermal convection resulting from Joule heating in larger bed separation units. CFFE employs a series of evenly spaced Teflon capillary tubes *within* the separation chamber which act as very efficient heat exchangers, resembling the heat exchanging ability seen in CE. Cooling water is then pumped through the capillary tubes while electrophoresis takes place outside the tubes. By layering rows of capillary tubes, the thickness of the separation bed can be increased without concern for Joule heating. It has been shown that the cooling tubes themselves do not appear to adversely affect the quality of separations(3).

CFFE separations are based on differences in electrophoretic mobility of analyte ions. When the sample ions are placed in an electric field, the ions migrate in a direction parallel to the electric field, with cations moving toward the cathode and anions moving toward the anode. The velocity, υ_i, of a given ion is described by

$$\upsilon_i = \mu_i E \qquad (1)$$

where μ_i is the electrophoretic mobility and E is the potential gradient. The electrophoretic mobility of a particular ion in solution is expressed as

$$\mu_i = |Z_i| q_o/6\pi\eta r_i \qquad (2)$$

where Z_i is the ion charge, q_o is the elementary charge, η is the viscosity of the medium, and r_i is the ion radius(5). Clearly, ionic mobility in a given solution is a function of charge-to-size ratio, resulting in varying ion mobilities and velocities.

In CFFE, sample is continuously fed into a curtain of buffer or suitable electrolyte at the top of the separation chamber. The carrier and sample flow under low pressure created by a peristaltic pump through the length of the chamber, perpendicular to the applied electric field, with velocity β. Simultaneously, the solute ions undergo electrophoretic motion parallel to the electric field, as described earlier. When carrier and separated analytes reach the bottom of the chamber, they exit through a series of Teflon tubes and are collected continuously as fractions, which are then analyzed appropriately. For a given set of conditions, an analyte ion will be deflected from its point of entry into the separation chamber by an angle Θ, the tangent of which is simply the ratio of the

electrophoretic velocity, υ_i , to the buffer velocity, β. This angle of deflection, Θ, is described by

$$\tan \Theta = \mu_i i/A\kappa\beta \qquad (3)$$

where i is the electric current through the chamber, A is the cross-sectional area of the chamber, and κ is the specific conductance of the carrier buffer solution(6). It can be shown that if the potential gradient is constant across the CFFE chamber, the angle of deflection is independent of carrier solution conductivity. The above relations assume thermal homogeneity and plug flow of the carrier solution through the CFFE bed; thermal instabilities and laminar flow produce deviation in predicted analyte ion migration.

Band Dispersion in Capillary Free-Flow Electrophoresis

A number of different phenomena have been identified which contribute to flow distortions in FFE(4), including hydrodynamic, electrodynamic, and electrohydrodynamic distortions.

Hydrodynamic distortion results from a parabolic or laminar flow profile of the carrier solution and gives rise to a sample band having broadened crescent shapes instead of the desired linear ribbon shape(7,8). Hydrodynamic distortion is minimized by employing a narrow diameter sample band at the point of introduction or by increasing the distance between the chamber walls. However, increasing the chamber size makes removal of joule heat more difficult; in this respect, CFFE is superior to conventional FFE, since the capillary cooling scheme allows for increased chamber dimensions.

Electrodynamic distortion is a consequence of electroosmotic flow which produces a net migration of solute ions toward the cathode wall(9,10). Since the ions closest to the cathode are affected to a larger degree than those toward the middle of the chamber, the solute band again develops a crescent shape. Electrodynamic effects in CFFE should be diminished with respect to conventional FFE, as a result of having multiple rows of Teflon capillaries in the separation chamber. The capillaries are expected to diminish the distance over which the analytes experience electroosmotic flow(3).

Electrohydrodynamic distortion will occur when the sample stream and carrier solution have differing conductivities(10-12). If the sample stream is less conductive than

the carrier, the sample band will be broadened in the direction perpendicular to the applied field, toward the front and rear walls of the FFE chamber. If the sample is more conductive than the carrier, the sample band will be broadened parallel to the electric field. This type of distortion is alleviated by minimizing differences in conductivity between sample and carrier solutions.

The presence of these distortive phenomena may be deleterious to the success of the desired separation. Unfortunately, the nature of the CFFE separation is such that one can only know with certainty the initial and final distribution of the analytes with respect to the direction of electrophoretic motion in the cell. Only in those cases where one or more of the compounds being separated is sufficiently colored in solution, can the progression of the analyte through the separation chamber be monitored visually. This can often provide an indication of the presence of distorted carrier flow for a given set of operating parameters. In most cases however, the analytes in solution are colorless or too dilute to be followed visually. In this situation, one simply attempts to avoid employing parameters which may contribute to such distortions. In fact, it is possible that the flow distortions which may contribute to band dispersion under a given set of operating parameters may interfere destructively, in effect canceling each other out. In such a case, the operator may never suspect their presence.

EXPERIMENTAL

The design of the separation device employed in this work (EM Separations Technology) has been described in detail previously(3) (Figure 1). This particular unit, however, is equipped with 48 outlet tubes for fraction collection, rather than 36. Additionally, only 3 sample inlet ports are present at the top of the separation bed, as opposed to 7 on the initial prototype unit.

Tap water, passed through a particulate filtration unit prior to use, was employed as coolant. The coolant enters through the bottom coolant chamber, flows upward through the cooling capillaries, and exits through the top coolant chamber. All carrier, sample, and electrode rinse electrolyte solutions were supplied by three separate peristaltic pumps. Regenerated cellulose acetate dialysis membranes were used to separate the electrode chambers from the main separation cell. Electrodes consist of Pt-coated Nb gauze; typical operating voltages are 100-400 V @ 100-600 mA.

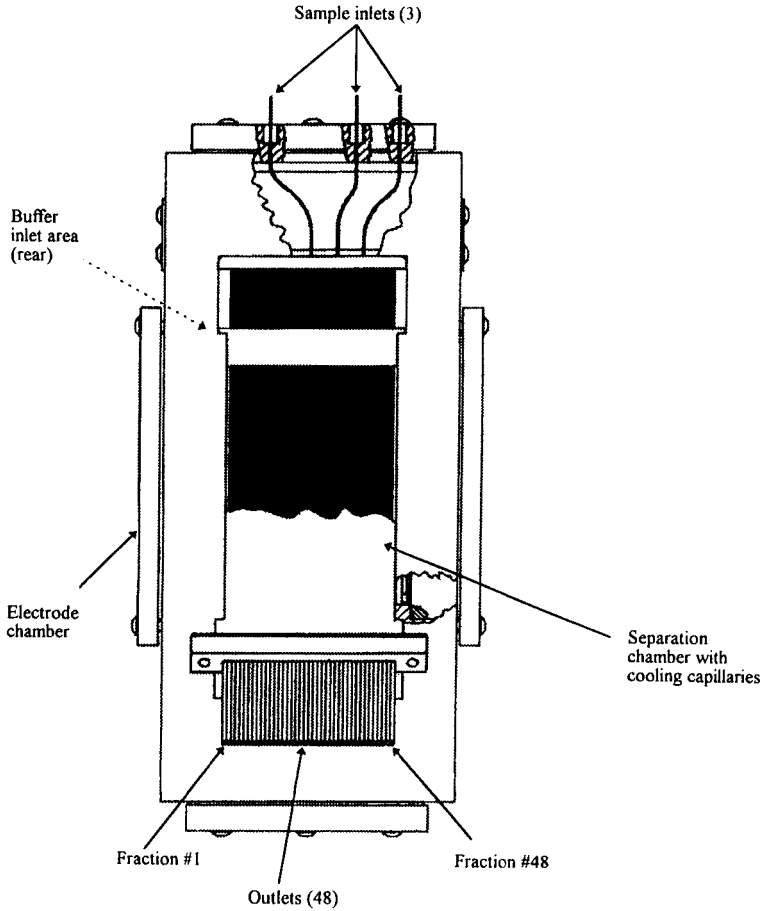

Fig. 1. Capillary free-flow-electrophoresis separation system.

Other Equipment

Inductively coupled plasma atomic emission spectroscopy (ICPAES) analyses of fractions were performed on a Perkin-Elmer Plasma 40 Emission Spectrometer. Inductively coupled plasma mass spectrometry (ICPMS) determinations were performed on a Perkin-Elmer Sciex Elan 500 system. Operating conditions for all ICPMS work were optimized for the particular element in question, using established procedures(13).

All pH measurements were made using a Sargent-Welch Model 6050 pH meter with a combination electrode.

Cobalt Species Separation

The separation of $[Co^{III}(sepulchrate)]^{3+}$ and $[Co^{III}(CN)_6]^{3-}$ was performed in zone electrophoresis (ZE) mode using 5mM potassium phosphate carrier buffer at pH 6.95. A cobalt stock solution was constructed by dissolving a sufficient amount of cobalt (III) sepulchrate trichloride (Aldrich, 95%) and potassium hexacyanocobaltate (Alfa Aesar, 90+%) in a portion of carrier buffer to give a cobalt concentration of approximately 100 mg/L in *each* species. The CFFE sample solution was a 1:20 dilution of the stock with additional 5 mM phosphate buffer. Sample was introduced at the center inlet port, approximately above Fraction 24, at a rate of 0.20 mL/minute. The carrier buffer flow rate was 13.7 mL/minute. The electrode rinse solutions were 15 mM dibasic potassium phosphate (pH 9.4) for the anode and 15 mM potassium phosphate buffer (pH 3.1) for the cathode. In these and all subsequent experiments, a minimum of 15 minutes equilibration time was allowed between initiation of the electric field/sample introduction, and the beginning of fraction collection. This insured that the collected fractions were representative of the analyte distribution at the bottom of the separation chamber. The cobalt content of each fraction was determined by ICPMS using copper as internal standard.

Chromium Separation

$[Cr^{III}(H_2O)_6]^{3+}$ and $Cr^{VI}O_4^{2-}$ were successfully separated by CFFE using a 10 mM perchloric acid/ ammonium perchlorate buffer solution at pH 2.3 and an electric field potential of 175 V. The flow rate of the carrier was 12.5 mL/minute. The sample, a solution of potassium chromate (Mallinkrodt Analytical Reagent) and chromium nitrate 9-hydrate (Baker Analyzed Reagent) dissolved in carrier buffer, was constructed such that each species would contribute approximately 100 mg/L chromium to the solution. Sample solution was introduced into the center of the flowing carrier stream at a rate of 0.20 mL/minute. The cathode rinse solution was 30 mM aqueous $HClO_4$ and the anode rinse was 30 mM $HClO_4$/ NH_4ClO_4, adjusted to pH 3.71 with 6 M aqueous ammonia. As a result of the electrode reactions, hydronium ion is generated at the anode and hydroxide ion is produced at the cathode. These reactions are capable of altering the pH

and conductivity of the nearby carrier solution, effectively creating a pH gradient across the main CFFE chamber which may interfere with the separation. This is of concern when working with analytes that are stable only in a particular pH range, or whose chemical forms are pH dependent, as in the case of the chromium (III) hexaaquo ion, which has a pK_{a1} of ~4. Therefore, the pH of each electrode rinse solution is selected so as to maintain essentially constant pH across the carrier buffer. The required pH and ionic strength of each rinse solution are determined by monitoring the pH of collected fractions and making adjustments as dictated by this information. Poor resolution and excessively broad analyte bands are symptomatic of inadequate control of pH(3).

Cr was determined in the collected fractions by ICPAES using the 205.552-nm CrII line.

Arsenic Separation

The incomplete resolution of three arsenic-containing compounds via CFFE was observed using a 10 mM potassium phosphate carrier buffer solution at pH 7.71, at a flow rate of 10. mL/minute. The electric field potential was 325 V. A stock solution of the analytes of interest was formulated by dissolving sodium arsenite (Baker Analyzed Reagent), sodium arsenate, dibasic, heptahydrate (Baker), and dimethylarsinic acid (Sigma) in a portion of the carrier buffer solution. A 5-fold dilution with additional buffer solution gave a CFFE sample solution in which the overall As concentration was 45 mg/L, contributed to equally by the three compounds. To facilitate the desired separation, the pH of the buffer was selected such that arsenite would be a neutral species, dimethylarsinic acid would be predominantly deprotonated and have a charge of -1, and arsenate would be a fully deprotonated dianion. The sample was introduced through the offset inlet located approximately over Fraction 32, at a flow rate of 0.20 mL/minute. The electrode rinse solutions used were 15 mM monobasic potassium phosphate (pH 4.76) and 15 mM potassium phosphate buffer, adjusted to pH 9.48 with a few drops of 6 M aqueous ammonia, for the cathode and anode, respectively. As in the chromium speciation, maintenance of constant pH across the carrier buffer was necessary to insure that the chemical forms of the three arsenic species did not change during the separation.

The arsenic concentration of each fraction was determined by ICPMS using nickel as internal standard.

RESULTS AND DISCUSSION

Figure 2A represents the separation pattern of $[Co^{III}$ (sepulchrate)$]^{3+}$ and $[Co^{III}(CN)_6]^{3-}$ using an electric field potential of 125 V (140 mA resulting current). The aforementioned CFFE parameters yielded optimal separation; at higher electric field potential or lower carrier buffer flow rate, a portion of the hexacyanocobaltate ions migrated through the dialysis membrane into the anode chamber, as indicated by an incomplete cobalt band in the resulting separation profile.

A series of separations were performed to determine the effect of varying electric field potential on the resolution of the two cobalt species. Figure 2B illustrates the results. The figure indicates that for intermediate potentials, 75 to 175 V, the relationship between resolution and electric field potential appears linear, as expected based on theory. However, at the extreme of 225 V, slight deviation is observed. At 225 V, the hexacyanocobaltate concentration was a maximum in fraction 45, and failed to return to zero concentration before reaching the membrane dividing Fraction 48 from the electrode chamber. It is very likely that a portion of this hexacyanocobaltate actually migrated through the membrane into the anode chamber; this phenomenon has been observed extensively in prior experiments. As a result, this analyte band appears to have a narrower base width than expected, based on the results of the trials at other electric field potentials. This smaller-than-expected bandwidth gives rise to the positive deviation in resolution at 225 V, as observed in the plot. In addition, it is feasible that hydrodynamic distortion, band spreading resulting from differences in migration time of solute ions caused by parabolic flow profile, may also be responsible. Hydrodynamic distortion of carrier buffer flow results in a reduced rate of flow near the walls of the separation chamber. As a result, solute ions in this region will experience a reduced flow rate, spend more time in the electric field, and therefore migrate proportionally further than those analyte ions near the center of the cell. In this experiment, the consequence is a larger-than-expected resolution, as the hexacyanocobaltate approaches the wall of the separation chamber closely. In the absence of an applied field, it is expected that no electrophoretic motion of analyte ions is observed, and hence a resolution of zero should be observed. However, it is clear in Figure 2B that a best fit line through the experimental points would not intersect the ordinate at zero. Currently, no explanation is available for this observation, although it appears dependent on the presence of the applied electric field.

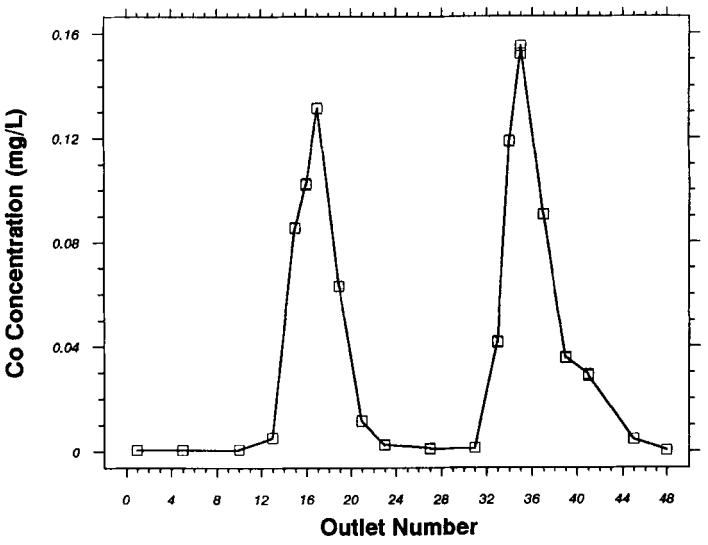

Fig. 2A. Separation profile of $[Co^{III}$ (sepulchrate)$]^{3+}$ (for left band) and $(Co^{III}(CN)_6]^{3-}$ conditions: 125 V; 5mM KH_2PO_4/K_2HPO_4 carrier buffer, pH 6.95, 13.7 mL/min flow.

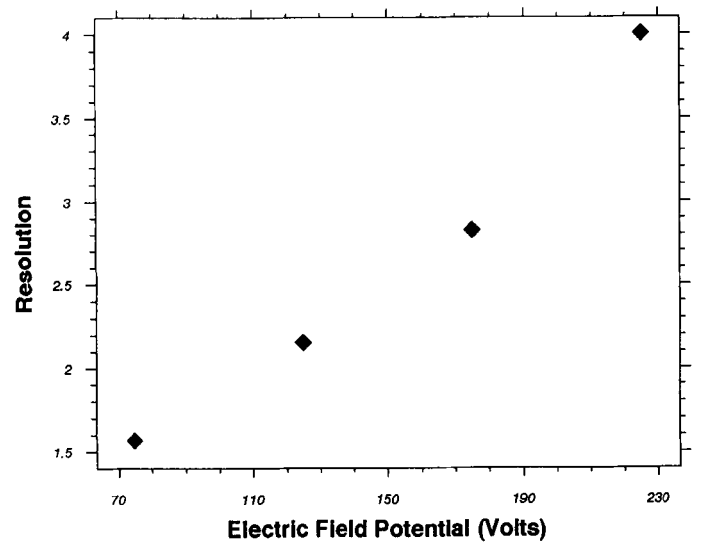

Fig. 2B. Plot of resolution versus electric field potential for CFFE separation of $[Co^{III}$/sepulchrate$]^{3+}$ and $[Co(CN)_6]^{3-}$.

As is evident in Figure 3, baseline resolution of $[Cr^{III}(H_2O)_6]^{3+}$ and $Cr^{VI}O_4^{2-}$ is achieved at an electric field potential of 175 V, while insuring that each analyte remains completely in the main separation chamber of the CFFE unit. It is immediately apparent that the baseline widths of the chromium bands depicted in the figure are significantly greater than those observed in the cobalt separation. This is likely a consequence of the larger sample loading employed for chromium, as required to successfully monitor the fractions using the Plasma 40 emission spectrometer, which gives poorer sensitivity than the Elan 500 mass spectrometer. This illustrates the electrohydrodynamic distortive effect, which was discussed earlier. When the sample conductivity is greater than the conductivity of the surrounding buffer, the sample band is flattened out in the direction of the electrodes, giving rise to broadened analyte bands in the CFFE concentration profile. Attempts to replicate the separation indicate that although the band shapes and absolute positions may vary slightly, within 2 or 3 fractions from run to run, the resolution of the two species remains fairly constant.

Figure 4 depicts the fraction profile for the incomplete separation of arsenate, arsenite, and dimethylarsinic acid. Attempts were made to improve the resolution of the separation by manipulation of carrier buffer flow rate and electric potential. However, these efforts led to loss of one of the three bands in the resulting CFFE profile. The probable assignment of the three analyte bands (proceeding from Fraction 1 to Fraction 48) is arsenate, dimethylarsinic acid, and arsenite. The task of proving with certainty this band assignment is complicated by the fact that from run to run, the absolute position of a single analyte introduced into the CFFE appears to vary slightly. Clearly, then, relative rather than absolute position will be useful in making assignments of a series of closely space analyte bands, as seen here. Other techniques, such as UV/Vis spectrophotometry, are being investigated for the unequivocal identification of the major component of each analyte band.

CONCLUSIONS

The successful use of CFFE in performing continuous separations of small Co, Cr, and As-containing species has been demonstrated. Complete resolution is obtained in those cases where two analytes of interest are present, as in the speciations of cobalt and

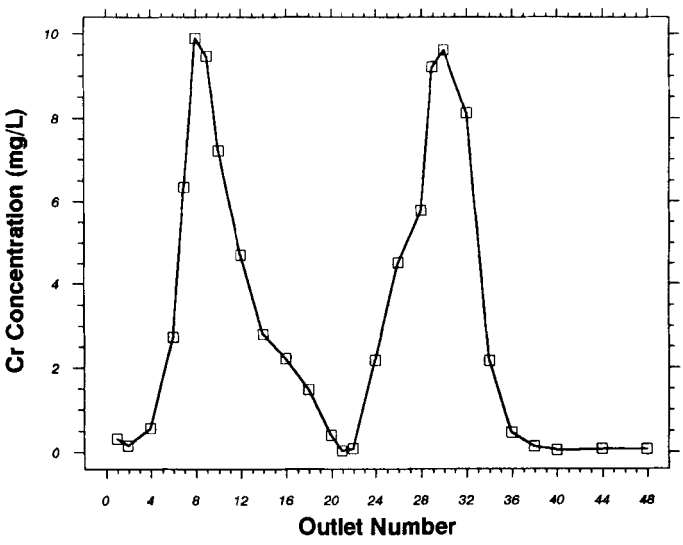

Fig. 3. Separation profile of $[Cr^{III}(H_2O)_6]^{3+}$ (for left band) and $Cr^{VI}O_4^{2-}$; conditions: 175 V; 10mM $HClO_4/NH_4C1O_4$ carrier buffer; pH 2.3; 12.5 mL/min flow.

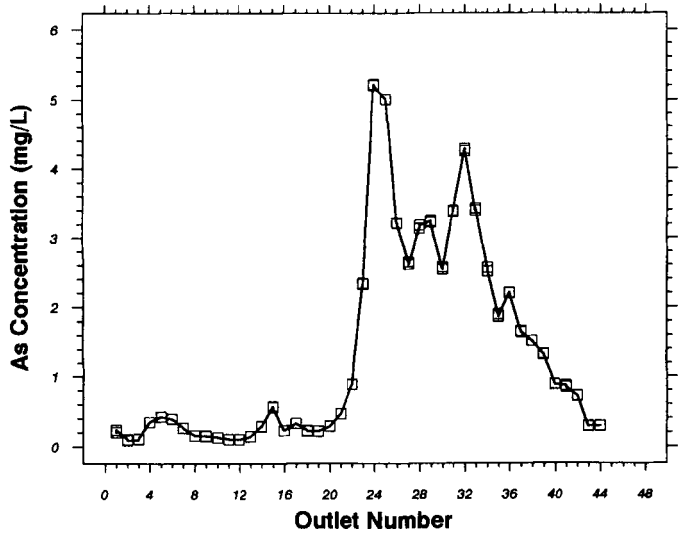

Fig. 4. Separation profile of arsenic species as $HasO_4^{2-}$, $(CH_3)_2$ As(O)O$^-$, and $HasO_2$ (from left to right); conditions; 325 V; 10mM KH_2PO_4/K_2HPO_4 carrier buffer, pH 7.71, 10.0 mL/min flow.

chromium. If three sufficiently similar analytes are present, as in the arsenic experiment, complete resolution is difficult employing the current CFFE design described herein. This work supports earlier findings that the use of a capillary bed acting as an internal heat exchanger effectively dissipates Joule heating in FFE, which is crucial to the elimination of thermal convection in scaled-up FFE design.

Future improvement in CFFE design could involve changes in the dimensions of the separation chamber to achieve improved separation of analytes having very similar electrophoretic mobilities. Additionally, the question arises concerning the effect of the fraction tubes mounted at the bottom of the separation chamber on the flow of buffer and analytes out of the separation chamber. Modification of the mounting technique and the geometry of the ends of the tubes are two issues worthy of investigation.

Overall, the advantages of continuous sample throughput, unattended operation, and gentle separation conditions make CFFE a potentially attractive technique in any field of chemistry which can make use of the electrophoretic properties of ionic analytes.

ACKNOWLEDGEMENTS

Portions of this work were supported through the Society for Analytical Chemists of Pittsburgh's Starter Grant Award (to MEK). The ICPAES and ICPMS instruments located at John Carroll University are donations of VWR Corp. for which the authors are grateful.

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