Proc. Estonian Acad. Sci. Chem., 2004, **53**, 2, 65–74
https://doi.org/10.3176/chem.2004.2.02

Speciation of chromium using wide-bore capillary electrophoresis with electrothermal atomic absorption spectrometry detection

Ruth Kuldvee, Aleksei Zaidentsal, Anu Viitak*, Maili Treumann, and Mihkel Kaljurand

Department of Chemistry, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia

Received 15 December 2003, in revised form 20 February 2004

Abstract. The development of a new method for chromium speciation using capillary electrophoresis–electrothermal atomic absorption spectrometry (CE–ETAAS) is described. For that purpose a simple capillary electrophoresis device enabling simultaneous separation and concentration of different chromium species has been designed and constructed. The device proved suitable for separating Cr(III) and Cr(VI) in aqueous samples. The sample amounts were sufficient for off-line collection and subsequent off-line detection with ETAAS. The influence of sample and background electrolyte pH values on the analytes' separation and recovery was investigated. The separation time and sample zone length were optimized. The limit of detection for both analytes was 1 ng/mL. RSD of the experiments was about 10%.

Key words: chromium speciation, wide-bore capillaries, preparative capillary electrophoresis, stacking.

INTRODUCTION

Thanks to its high selectivity and low detection limits atomic absorption spectrometry (AAS) is a widely used technique for determination of total metal content of various complex samples. One of the most serious limitations of the method when applied on natural samples is its disability to separate different forms of the metal. However, sometimes the knowledge of in what form the metal was in the original sample is crucial, especially when one form of the metal is significantly more poisonous (or more useful) than the other. A well-known case is chromium. Inorganic chromium is one of the primary quality indicators in

* Corresponding author, anuv@edu.ttu.ee

natural water analysis. The toxicity of chromium depends critically on its oxidation state. Chromium(VI) is known to be toxic and carcinogenic [1], but chromium(III) is considered essential to mammals, its deficiency may result in glucose intolerance in diabetics, arteriosclerosis, heart disease, depressed growth, obesity, and tiredness [2, 3].

For elemental speciation of chromium in aqueous samples various techniques have been described in the literature. Currently preferred approaches are the so-called hyphenated techniques where a high performance separation method is coupled with an element-selective detector [4]. The most frequently used separation methods have been liquid chromatography [5–8] and flow injection analysis related techniques [9–11]. Capillary electrophoresis (CE), which is also known as a powerful separation technique, has been used for metal speciation mainly together with very sensitive and selective but costly detectors such as inductively coupled plasma spectrometry–mass spectrometry [12–14]. For low budget labs the latter is seldom available. The use of less sensitive detection systems such as flame AAS is restricted because of the rather high detection limits of CE. The latter is due to the small dimensions of the capillary limiting the sample volumes analysed. Another limitation for the hyphenation of CE and AAS is the rigidness of commercial devices of CE for any kind of instrumental modifications. According to the authors' knowledge only two groups have studied speciation of chromium by combining CE principles (i.e. the separation is based on different mobilities of ionic species in electric field) with AAS. He et al. [15, 16] constructed a rather complicated CE device to separate and pre-concentrate Cr(III) and Cr(VI) simultaneously. Deforce et al. [17] constructed a preparative CE device for off-line connection with electrothermal atomic absorption spectrometry (ETAAS) using fused silica capillaries with i.d. of 75 μm. Their system enabled to collect a single fraction of the sample and due to the small dimensions of the capillary the concentration of the analyte was unavoidable.

We made a new approach: a preparative CE device that allows stacking the analyte during the separation was applied. The CE device is off-line hyphenated with ETAAS. To overcome the high detection limits of the method two approaches were applied. The use of wide-bore capillaries (i.d. 300 μm) instead of the conventional ones (i.d. 50–70 μm) allows introduction of several times larger sample volumes (over 120 μL) and collection of fractions of sample for off-line ETAAS detection. The CE unit is designed to simultaneously separate and concentrate Cr(III) and Cr(VI). The separation and concentration are based on the fact that Cr(III) exists in aqueous solutions predominantly in cationic form (Cr^{3+}) and Cr(VI) in anionic form ($Cr_2O_7^{2-}$), which migrate in electric field towards cathode and anode, respectively. If the ionic strength of the sample is at least 10 times lower than that of the background electrolyte (BGE) electrostacking occurs, i.e. the sample ions stack on the border of the sample and BGE zones resulting in the concentration of the sample. ETAAS is employed as an analytical detector for the measurement of both Cr(III) and Cr(VI) in standard solutions and in wastewater samples.

EXPERIMENTAL

Instruments

A Philips PU 9100X atomic absorption spectrometer with a PU9390X electro-thermal atomizer was employed as the detector in chromium determination. The measurement wavelength was 429 nm, bandwidth 0.5 nm, and lamp current 10 mA. Sample (20 μL) was injected into a pyrolytic graphite tube. The furnace program was set up as follows: drying (120 °C for 10 s), charring (500 °C for 20 s), ashing (1400 °C for 10 s), atomization (2600 °C for 2 s), and cleaning (2800 °C for 2 s).

A schematic diagram of the CE separation device is shown in Fig. 1. A Teflon tube of 0.3 mm i.d. was used as the separation and pre-concentration capillary. A simple gas displacement pump consisting of pressurized G4 type glass bottles was constructed for introducing the sample and the solution of electrolyte into the capillary. The power supply constructed in the lab allows delivering up to +22 kV.

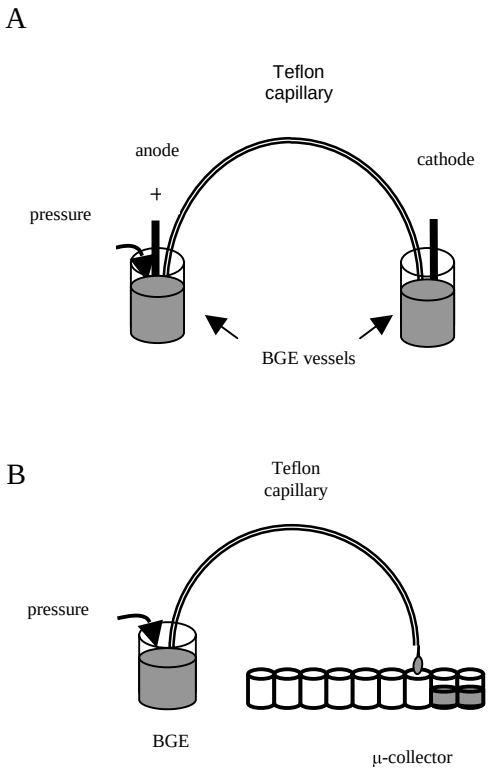

Fig. 1. A schematic picture of the preparative CE device. A – the separation step; B – the collection of sample fractions.

Chemicals

All standard solutions and reagents were prepared using Milli-Q water. All the chemicals were of analytical reagent grade. The stock solutions of Cr(III) and Cr(VI) were prepared of $Cr(NO_3)_3 \cdot 9H_2O$ (Fluka) and $K_2Cr_2O_7$ (Fluka), respectively. HNO_3 concentrated solution was from Merck, Suprapur. The inter-calibration drinking water samples were received from the Estonian Research Centre.

The choice of the pH of BGE and sample solutions

It is known from the literature that the oxidation state of chromium depends on the pH value of the environment [18]. It is also known that Cr(III) tends to hydrolyse and precipitate at pH values higher than 4.5. The pH range of this work was chosen to be less than 4.5 to avoid the precipitation of Cr(III) [13]. If not stated otherwise the BGE used in CE separation was 0.04 M HNO_3 solution (pH 2.5). The calibration solutions used for ETAAS determination were prepared by diluting Cr(III) and Cr(VI) stock solutions with diluted HNO_3 (for exact concentration, see the text). The calibration range was from 2 to 25 ng/mL.

Procedures

The choice of the length of the capillary

The inlet end of the capillary was inserted into the pressurized vessels. By pressurizing BGE or the sample vessel using carefully selected timing sequences, it was possible to fill the capillary with sample and BGE solutions with pre-set zone lengths. As the sample load in CE is in proportion to the inner diameter of the capillary the use of wide-bore capillaries would be advantageous. Otherwise the analysis of diluted samples would be problematic. On the other hand, the larger is the capillary inner diameter at the given length, the smaller is the liquid flow in the less restricted capillary and even tiny differences in liquid levels in the capillary inlet and outlet vials become significant creating additional pressure in the capillary, i.e. siphoning effects. These lead to the broadening of the zones and dilution of the sample. To minimize the siphoning effect either automated vial lifting [19] or use of restrictors in the capillary ends [20] has been used. In our case the siphoning effect was suppressed by using a relatively long capillary (4 m). This length was found to be enough to neglect the hydrodynamic flow caused by millimetre-range differences in capillary inlet and outlet vials.

Operation of the CE unit

To sandwich the sample between BGE zones the capillary inlet end was first placed into the BGE vessel and a portion of BGE solution was inserted by pressure (2×10^5 Pa) into the capillary. Next the pressure was released, the BGE vessel was replaced with the sample vessel, and the operation was repeated. Then the sample vessel was again changed for the BGE vessel and another portion of

Table 1. Description and duration of procedures of the CE unit

Step	Procedure	Duration
1	Filling and conditioning the capillary with BGE	5 min
2	Loading the sample	20–80 s
3	Loading the BGE	15–55 s
4	Switching on the high voltage; the separation step	5–50 min
5	Switching off the high voltage; applying pressure to the capillary and collecting fractions of sample to microvials	1 min
6	Washing the capillary with conc. HNO_3 solution	5–30 min

BGE solution, equal in volume to the first BGE portion, was inserted into the capillary. After that the pressure was released again, both ends of the capillary were immersed into BGE solutions, and high voltage was applied via electrodes mounted into BGE vessels. The separation step was carried out. After the desired separation time had passed the high voltage was switched off, the capillary outlet end was removed from the BGE vessel, and fractions of the capillary content were forced into a microcollector by applying pressure on the inlet end of the capillary. Collected fractions were measured off-line by ETAAS. Between the measurements the capillary was washed with concentrated HNO_3 solution to avoid deterioration of the samples due to possible adsorption/precipitation of chromium species on the capillary wall. Typical spans of operations are given in Table 1.

RESULTS AND DISCUSSION

Separation of Cr(III) and Cr(VI)

The separation and following detection are shown in Fig. 2. The zones of Cr(III) and Cr(VI) are clearly baseline separated. To confirm the peaks presented in Fig. 2c the pherograms of the samples consisting of only Cr(III) and Cr(VI) are displayed in Figs. 2a and b, respectively. It is also seen from Fig. 2 that the first and last fractions in the capillary are free from chromium. It is important not just to avoid the escaping of the analytes from the capillary and thus the loss of the sample. It is also important to keep the electrodes clean from chromium contamination. He et al. [15] used salt bridges for that purpose. However, in the present work a simpler approach was used. Instead of building salt bridges the electrodes were protected from chromate contamination by simply adjusting the ionic strength of the BGE at least 100-fold higher than that of the sample. Consequently the electric field strength in the sample zone was kept 100 times higher than in the BGE zone. The analytes were slowing down and stacking on the border of the BGE zone since the migration velocity of an analyte depends on the electric field strength of the environment. Thus the velocity of the analyte ions in the BGE zone was strongly reduced and it was practically excluded that chromate/chromium ions could reach inlet/outlet vials (and electrodes).

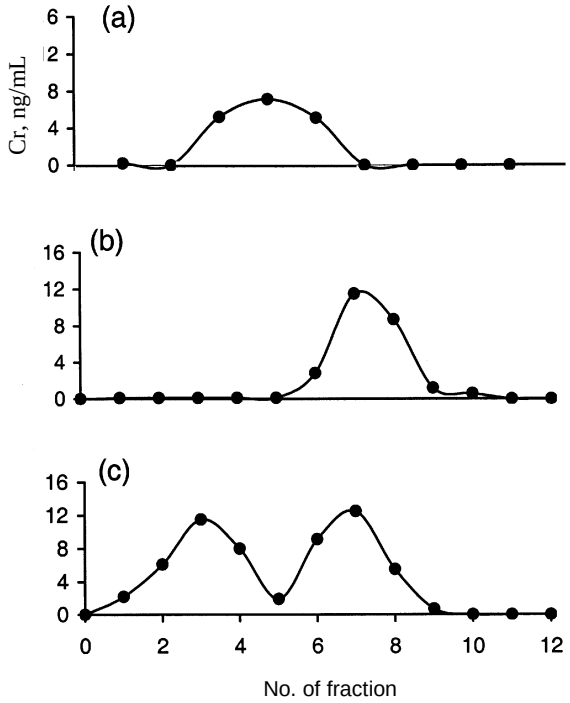

Fig. 2. Separation of Cr(III) and Cr(VI). Composition of the sample: (a) – 5 ng/mL of Cr(III) in diluted HNO_3 solution; (b) – 5 ng/mL of Cr(VI) in diluted HNO_3 solution; (c) – 5 ng/mL of Cr(III) and Cr(VI) each in diluted HNO_3 solution. Experimental conditions: the ionic strength of the sample is 0.005 mM, the ionic strength of the HNO_3 BGE is 0.5 mM. High voltage was applied for 40 min. The dimensions of the Teflon capillary: 0.3 mm i.d., 4.1 m length. The volume of the collected fraction is 22 μL. High voltage is 22.4 kV, current is 1–2 μA.

To optimize the separation of Cr(III) and Cr(VI) the duration of the analysis and the length of the sample zone were tuned. Analysis time of 40 min was chosen to have a baseline separation and to ensure a high zone maximum. Within 40 min the baseline separation was achieved as demonstrated in Fig. 2.

The length of the sample zone had a direct influence on the CE performance. Too short sample zone caused incomplete separation during 40 min as illustrated by Fig. 3a. On the other hand, the longer the diluted sample zone the weaker is the electric field in this zone, and therefore, the slower is the velocity of ions. This situation is demonstrated in Fig. 3d: no clear separation of analytes is obtained during 40 min. Figure 3c shows that under the given conditions the optimal sample zone length is 55–60% of the total capillary length (i.e. in our case approximately 2.6 m). The zones are baseline separated and the maximum of the peaks is the highest among the possible ones.

The analysis time is still rather long but as the purpose of the paper is to present the preliminary performance of the device the shortening of the analysis time (via shortening the capillary, etc.) was left for future studies.

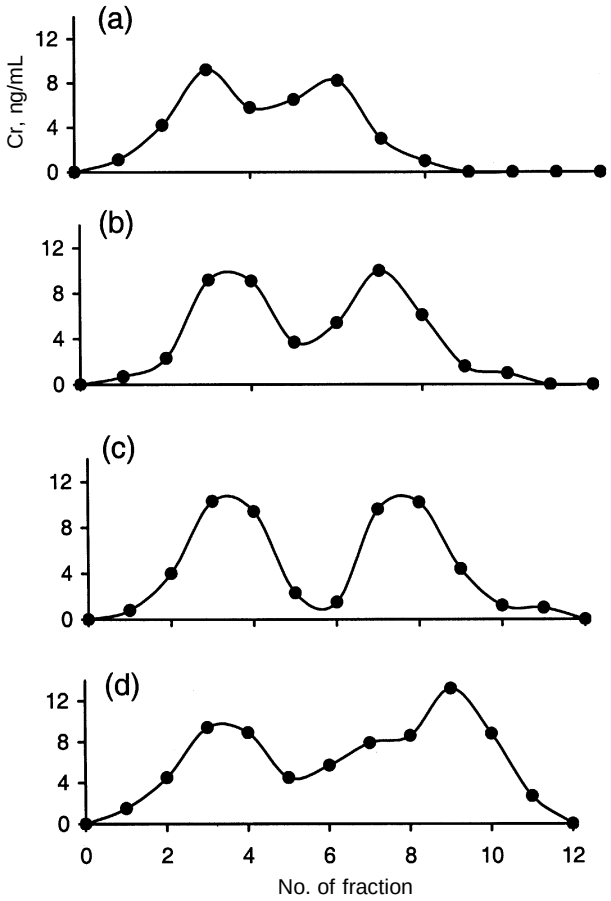

Fig. 3. The influence of the sample zone length on the separation of Cr(III) and Cr(VI). The proportion of the sample zone length from the total length of the capillary: (a) – 40%; (b) – 50%; (c) – 60%; (d) – 80%. For other conditions see Fig. 2.

Detection limits and linear detection range

The preliminary detection limits of the method were found to be 1 ng/mL for both Cr(III) and Cr(VI). The linear detection range was measured for the concentrations of interest, namely 2 to 25 ng/mL. The calibration graph equation was $y = -0.5043 + 1.006x$. The variation coefficient R was reasonably near to unit (0.9987). The overall average reproducibility was found to be 10%.

The detection limits could be significantly lowered by further optimizing the stacking conditions (via using shorter capillaries, higher voltage, and different BGEs). However, as the detection limits achieved are in most cases sufficient for real samples the reduction of detection limits was also left for future studies.

Sample stacking

The relationship between the known concentration of the original sample injected and the concentration of the sample actually measured was found to be reasonably linear. The variation coefficient was 0.9993. The measured concentration was found to be always double the original sample concentration. This indicates that at the chosen working conditions the stacking effects concentrate the sample 2 times.

Analysis of real samples

Although the analysis of real samples by the designed interface is not completely established on the routine basis yet, a few preliminary experiments were performed. In Fig. 4 it is demonstrated that the designed CE device enables indeed to perform chromium speciation. As it can be seen from Fig. 4 in the drinking water sample only Cr(VI) ions were present. The concentration of Cr(VI) was 3.2 ng/mL (CRM).

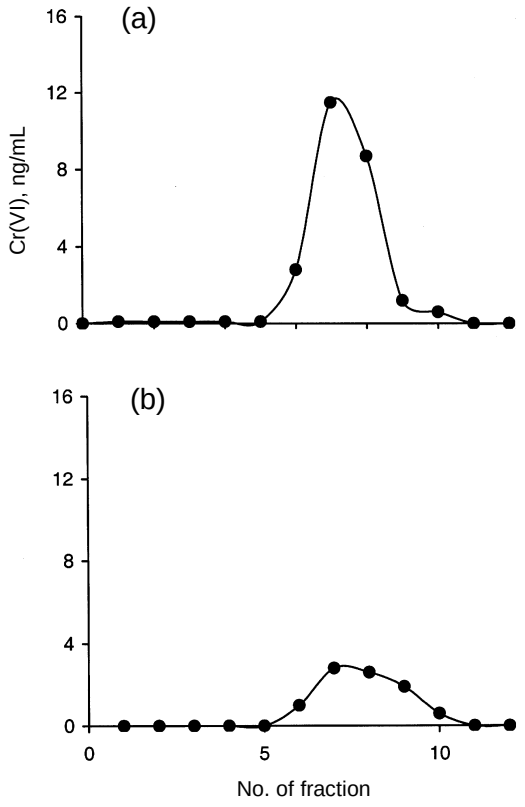

Fig. 4. The measurement of the drinking water sample. (a) – a standard solution of Cr(VI), concentration 12 ng/mL, (b) – a drinking water sample. The ionic strength of the BGE is 0.01 mM. For other conditions see Fig. 2.

CONCLUSIONS

The goal of the present work was to demonstrate that it is possible to separate different species of chromium by preparative CE using a simple wide-bore capillary. The volume of the capillary was large enough to allow collecting fractions for subsequent ETAAS detection. The present study proved that

(i) the separation of different chromium ions in a simple wide-bore Teflon capillary is possible,

(ii) the amount of the sample inserted into such a capillary is large enough to enable off-line detection of separated analytes by ETAAS,

(iii) the speciation device described provides a simple means to pre-concentrate and determine both Cr(III) and Cr(VI) in a single analytical cycle.

ACKNOWLEDGEMENTS

Professor C. H. Lochmüller from Duke University is thanked for donating the high voltage power supply. R.K., A.V., and A.Z. are grateful to the Estonian Science Foundation (grant No. 5618).

REFERENCES

1. Nriagu, J. O. & Nieboer, E. *Chromium in the Natural and Human Environment*. Wiley, New York, 1988.
2. Valcovic, V. *Trace Elemental Analysis*. Taylor and Frencis, London, 1975.
3. Dominick, B. *The People's Guide to Vitamins & Minerals*. Contemporary Books, Chicago, 1980.
4. Cámara, C., Cornelis, R. & Quevauviller, P. Assessment of methods currently used for the determination of Cr and Se species in solutions. *Trends Anal. Chem.*, 2000, **19**, 189–194.
5. Powell, M. J., Boomer, D. W. & Wiederin, D. R. Determination of chromium species in environmental samples using high pressure liquid chromatography direct injection nebulization and inductively coupled plasma mass spectrometry. *Anal. Chem*., 1995, **67**, 2474–2478.
6. Tomlinson, M. J. & Caruso, J. A. Speciation of chromium using thermospray nebulization as sample introduction into inductively coupled plasma mass spectrometry. *Anal. Chim. Acta*, 1996, **322**(1–2), 1–9.
7. Ali, I. & Aboul-Enein, H. Y. Speciation of arsenic and chromium metal ions by reversed phase high performance liquid chromatography. *Chemosphere*, 2002, **48**(3), 275–278.
8. Posta, J., Alimonti, A., Petrucci, F. & Caroli, S. Online separation and preconcentration of chromium species in seawater. *Anal. Chim. Acta*, 1996, **325**, 185–193.
9. Paleologos, E. K., Stalikas, C. D., Tzouwara-Karayanni, S. M. & Karayannis, M. I. Selective speciation of trace chromium through micelle-mediated preconcentration coupled with micellar flow injection analysis–spectrofluorimetry. *Anal. Chim. Acta*, 2001, **436**, 49–57.
10. Cuesta, A., Todoli, J. L. & Canals, A. Flow injecton method for the rapid determination of chemical oxygen demand based on microwave digestion and chromium speciation in flame atomic-absorption spectrometry. *Spectrochim. Acta, Part B: At. Spectrosc.*, 1996, **51**,1791–1800.

11. Ruz, J., Rioz, A., Luque de Castro, M. D. & Valcárcel, M. Flow injection configurations for chromium speciation with a single spectrofotometric detection. *Anal. Chim. Acta*, 1986, **186**, 139–146.
12. Olesik, J. W., Kinzer, J. A. & Olesik, S. V. Capillary electrophoresis inductively coupled plasma spectrometry for rapid elemental speciation. *Anal. Chem*., 1995, **67**, 1–12.
13. Stewart, I. I. & Olesik, J. W. Investigation of Cr(III) hydrolytic polymerization products by capillary electrophoresis–inductively coupled plasma mass spectrometry. *J. Chromatogr*., *A*, 2000, **872**, 227–246.
14. Michalke, B. & Schramel, P. Hyphenation of capillary electrophoresis to inductively coupled plasma mass spectrometry as an element-specific detection method for metal speciation. *J. Chromatogr*., *A*, 1996, **750**, 51–62.
15. He, Y., Cervera, M. L., Garrido-Ecija, M. & de la Guardia, M. On-line bidirectional electrostating of chromium(III) and chromium(VI) for flame atomic absorption spectrometry determination. *Anal. Chim. Acta*, 2000, **421**, 57–65.
16. He, Y., Cervera, M. L., Pastor, A. & de la Guardia, M. Determination of chromium(III) and chromium(VI) in mineral water by bidirectional electrostating and electrothermal atomic absorption spectrometry. *Anal. Chim. Acta*, 2001, **447**, 135–142.
17. Deforce, D. L., Kokotos, G., Esmans, E. L., De Leenheer, A. & Van den Eeckhout, E. G. Capillary electrophoresis and electrochromatography: preparative capillary zone electrophoresis in combination with off-line graphite furnace atomic absorption for the analysis of DNA-complexes formed by a new aminocoumarine platinum(II) compound. *Electrophoresis*, 1998, **19**, 2454–2458.
18. Yang, W.-P., Zhang, Z.-J. & Dengm, W. Simultaneous, sensitive and selective on-line cheminoluminescence determination of Cr(III) and Cr(VI) by capillary electrophoresis. *Anal. Chim. Acta*, 2003, **485**, 169–177.
19. Jussila, M., Palonen, S., Porras, S. P. & Riekkola, M.-L. Compensation of siphoning effect in nonaqueous capillary electrophoresis by vial lifting. *Electrophoresis*, 2000, **21**, 586–592.
20. Yin, H., Keely-Templin, C. & McManigill, D. J. Preparative capillary electrophoresis with wide-bore capillaries. *J. Chromatogr*., *A*, 1996, **744**, 45.

Kroomi eri vormide spetsifitseerimine suure diameetriga kapillaari elektroforeesi ja aatomiabsorptsioonspektrofotomeetria abil

Ruth Kuldvee, Aleksei Zaidentsal, Anu Viitak, Maili Treumann ja Mihkel Kaljurand

On uuritud laboris konstrueeritud preparatiivse seadme võimalusi ning selle sobilikkust kroomi eri vormide spetsifitseerimiseks. Detektorina kasutati elektrotermilist aatomiabsorptsioonspektrofotomeetriat. Kapillaaridena kasutati teflon- ja kvartskapillaare.

Kroomi eri vormide Cr(III) ja Cr(VI) näitel katsetati seadme rakendamist raskmetallide spetsifitseerimiseks. Proovi ioonide hoidmiseks kapillaaris leiti, et elektrolüüdilahus peab olema proovist vähemalt 100 korda suurema ioontugevusega ja tsooni pikkusel on analüüsiaja pikkusele oluline mõju. Optimaalseks pikkuseks on 50–60% kapillaari üldpikkusest.