

Multicommutation flow analysis with chemiluminescence detection: application to the chromium(III) determination*

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ABSTRACT

The study deals with the application of multicommutation flow analysis for determination of Cr(III) concentration in environmental samples at a trace level. The detection method was based on the luminol-H₂O₂ chemiluminescence reaction in basic aqueous solution using Cr(III) ions as a catalyst. The experimental part of investigation focused on the optimization of equipment conditions, i.e. reagent flow rate, injected sample volume and the work of solenoid valves. The specificity of the method was tested with respect to selected heavy metal ions. The presented method allowed determination of Cr(III) over the range from 2.5 to 50ng·cm⁻³, with a low detection limit

(under optimum conditions: 0.15ng·cm⁻³). Multicommutation is one of the best methods of analytical procedure automation. The main advantages of multicommutation method, compared with classical flow injection analysis (FIA), is the ability to achieve more repeatable analytical signal, which in turn allows to improve the method precision (relative standard deviation, RSD, was 1.8% for 10 determinations of 20ng·cm⁻³ Cr(III)). Moreover, it is excellent from the point of view of environmental protection because it offers very low reagent consumption: luminol 0.26; H₂O₂ 2.16; sample 0.15 (cm³ per peak). The sampling rate was about 90 samples·h⁻¹.

INTRODUCTION

The oxidation state of an element can be an important factor influencing its bioavailability and toxicity. Chromium ions exist in natural water and soil in two states: Cr(III) and Cr(VI). Chromium(III) is an essential trace element, required for the maintenance of normal glucose, lipid and protein metabolism, plays a role in various enzyme reactions. In contrast, water soluble chromium(VI), in the form of CrO₄²⁻ or Cr₂O₇²⁻, is highly irritating and toxic to humans and animals (Nriagu and Nieboer 1988). Therefore, the analysis of chromium speciation in environmental samples has become very important. The development of analytical methods that can determine chromium at trace level and distinguish between chromium(VI) and chromium(III) remains under the research all the time.

A number of papers have described analytical procedures for the determination of chromium in biological samples (Escobar et al. 1998), environmental water (Moliner-Martinez et al. 2003; Powell et al. 1995) and for chromium

speciation (Gammelgaard et al. 1997; Girard and Hubert 1996; Panssar-Kallio and Manninen 1996). The methods most frequently used for the total chromium determination is atomic absorption spectrometry (AAS) (Girard and Hubert 1996) and UV-Vis spectrophotometry (Andersen 1998). However, for direct determination of the chromium species, preconcentration of analyte from the matrix prior to measurement is often essential. Another solution is to apply enough sensitive detectors. A sufficiently sensitive detector system is, for example, inductively coupled plasma emission mass spectrometer (ICP-MS) (Panssar-Kallio and Manninen 1996; Powell et al. 1995). Moreover, for the speciation analysis, especially in environmental or biological samples, additional step is necessary – a separation step. This is the reason of gas chromatography (GC) or capillary electrophoresis (CE) application (Chen et al. 2001; Yang et al. 2003). However, such instruments are not available in most laboratories. As an alternative, the sensitive chemiluminescence detection can be used (Gammelgaard et al. 1997).

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Application of chemiluminescence (CL) for the analysis of chromium in a natural water has been previously reported (Escobar et al. 1995; Gammelgard et al. 1997; Seitz et al. 1972; Yang et al. 2003). The method is based on the measurement of Cr(III)-catalysed light emission from luminol oxidation by hydrogen peroxide. The specificity of this reaction for Cr(III) is achieved in the presence of ethylenediaminetetraacetic acid (EDTA) as a masking agent. The formation of Cr(III)-EDTA complex is kinetically slow in comparison to that of the other metals, which also catalyse the reaction of luminol (Moliner-Martinez et al. 2003; Seitz et al. 1972). Cr(VI) does not catalyse the reaction at all. This method can be used for the speciation of both forms of chromium, Cr(III) and Cr(VI).

Chemiluminescence detection can be coupled with the flow injection analysis (FIA) (Escobar et al. 1993, 1995, 1998). In this combination chemiluminescence provides sensitivity and selectivity, whereas FIA provides rapid and reproducible sample injection and mixing of the reagents. These factors, together with low cost and simplicity, make the FIA-chemiluminescence combination very attractive.

In this paper, multicommutation flow technique has been applied for the determination of Cr(III) in water samples. Multicommutation is an effective alternative to classical FIA method for the insertion of sample and reagents into a carrier stream in flow analysis. Multicommutation uses discrete commutation devices, such as three way solenoid valves. This allows the design of a flow network that can be precisely computer-controlled and fully automated.

Multicommutation provides also other advantages (Catala Icardo et al. 2002; Rocha et al 2002), such as:

1. Miniaturization of flow assemblies. The small size of solenoid valves and electronic interfaces permits developments of compact and integrated system.
2. Reduction sample and reagent consumption. Volumes of few microlitres, which correspond to insertion times within the range of a second, can be inserted very precisely.
3. Increase in reproducibility as compared to FIA. Solenoid valves require minimal operator intervention.
4. Economy and simplicity. Solenoid valves can be switched on and off by a simple electrical pulse; a card inserted into the computer's motherboard governs the whole system. All operations can be controlled via user-friendly, flexible software developed in a common programming language (e.g. Turbo Pascal).

However, there are also some disadvantages of the multicommutation. The method is relatively new. There is limited commercial availability of electronic interfaces and software for controlling solenoid valves. As a result, the method is not very common.

The aim of this study was to apply the multicommutation flow analysis with chemiluminescence detection in order to determine the content of the Cr(III) ions in water samples.

Numerous parameters of the system were optimized in order to improve the sensitivity and repeatability of the method.

MATERIAL AND METHODS

Reagents

Luminol was purchased from Fluka (Buchs). All other chemicals used were obtained from POCH (Gliwice, Poland) and were of analytical grade. The stock solutions of reagents were stored at 4°C to avoid exposure to light and air. Working solutions were prepared daily by a dilution of respective stock solutions with water obtained from a Milli-Q (Millipore) water purification system.

Solutions

Chromium standard stock solution: $1\text{mg}\cdot\text{cm}^{-3}$ Cr(III); 0.7696g of $\text{Cr}(\text{NO}_3)_3\cdot 9\text{H}_2\text{O}$ was dissolved and diluted to 100cm^3 with water.

Luminol stock solution: $10^{-2}\text{mol}\cdot\text{dm}^{-3}$; 0.1772g of luminol was dissolved and diluted to 100cm^3 with Na_2CO_3 - NaHCO_3 buffer solution (pH=10.7). All working solutions were prepared by appropriate diluting of stock solution with Na_2CO_3 - NaHCO_3 buffer solution.

Hydrogen peroxide stock solution: $1\text{mol}\cdot\text{dm}^{-3}$; 10cm^3 of 30% H_2O_2 was diluted to 100cm^3 with water.

EDTA stock solution: $10^{-3}\text{mol}\cdot\text{dm}^{-3}$; 0.0931g of EDTA was dissolved and diluted to 250cm^3 with water.

Apparatus

A schematic diagram of the multicommutation network used in this work is shown on the Figure 1. Three way Colle-Parmer solenoid valves were used for mixing the reagents (luminol and H_2O_2 ; valve V1) and for introducing of the analyte to the stream (valve V2). A Masterflex peristaltic pump (P) was used to aspirate the reagents and sample. All flow lines were made of TYGON® 3603 tubing (1.42mm i.d.). A flow luminometer (KSP, Poland) equipped with a spiral flow-cell (FC) made from coiled PTFE tube of 1mm i.d. (length of 50cm) oriented in front of the photomultiplier (PMT) window was used for measurement of CL intensities.

Three-way solenoid valves worked as a switch between two states: ON and OFF (Figure 2, part a). While the valve was OFF, the carrier solution (e.g. H_2O_2 through V1; H_2O_2 or mixture of H_2O_2 and luminol through V2 (Figure 1)) was aspirated in the direction of detector. When the valve was ON, an electronic pulse of programmable length allowed the appropriate reagent to be inserted into the carrier (luminol by V1; sample by valve V2 (Figure 1)). The volume of sample inserted was proportional to the pulse length and could be altered by changing the profile of the insertion sequence. The work of the pump and solenoid valves was computer controlled with the program elaborated in our laboratory. The optimized work of the solenoid valves V1 and V2 is shown on the Figure 2, part b.

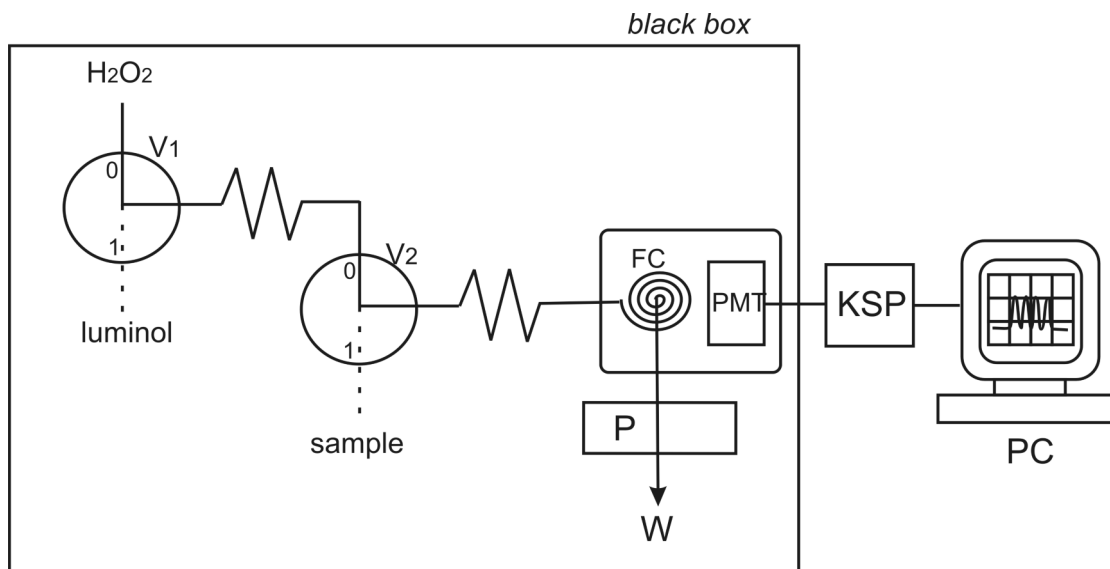


Figure 1. Schematic diagram of the multicommutation network used for Cr(III) determination: V1, V2 – solenoid valves; FC – flow cell; PMT – photomultiplier; P – peristaltic pump; W – waste; KSP – electronic device controlled by PC (personal computer).

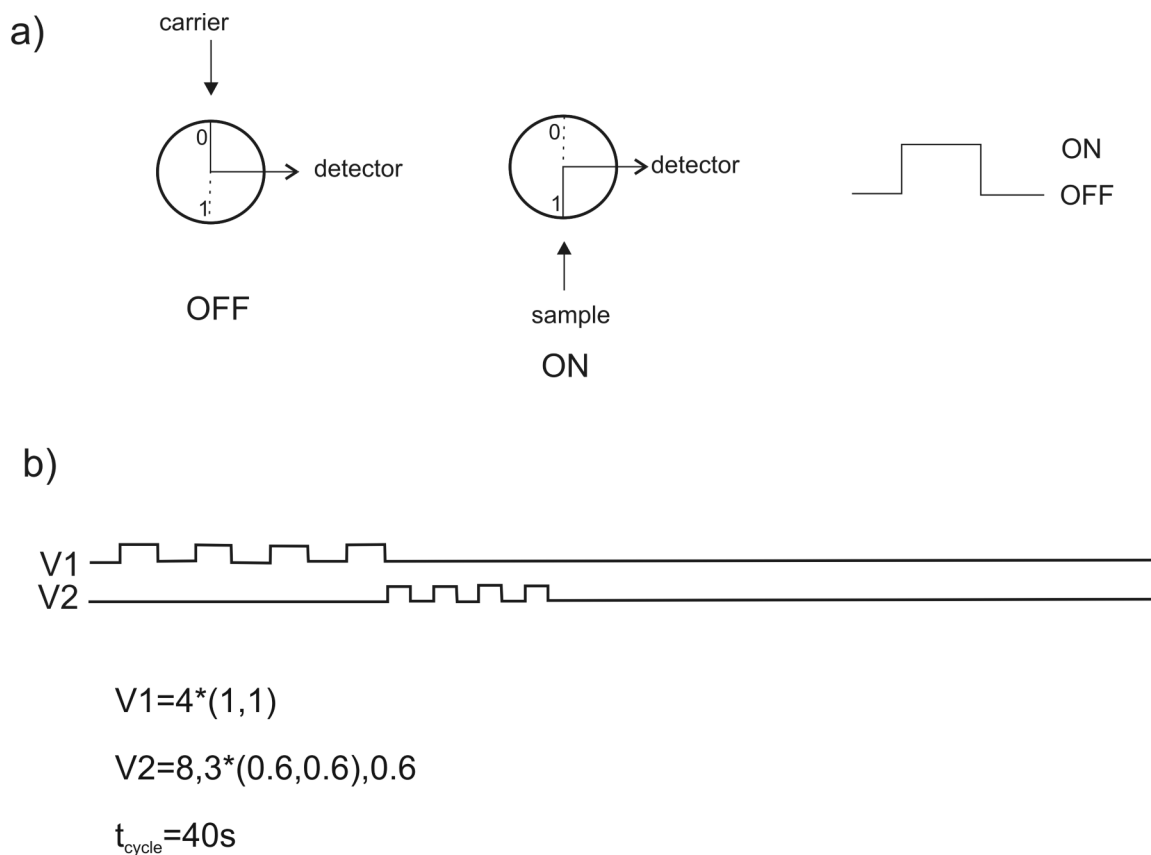


Figure 2. Time controlled insertion profiles of solenoid valves: (a) the valve status – ON or OFF; (b) the optimized program of solenoid valves V1 and V2; * – repetition of a time sequence (in brackets)..

RESULTS AND DISCUSSION

In order to establish the multicommutation parameters which gave the best analytical signal, a series of systematic optimisation procedures were performed with respect to their sensitivity and repeatability. The peak height, that means the difference of the peak signal from the noise (S-N), was taken into account.

Effect of instrumental variables

The experimental part of investigation focused on the optimization of equipment conditions, i.e. reagent flow rate, injection sample volume, and the work of solenoid valves.

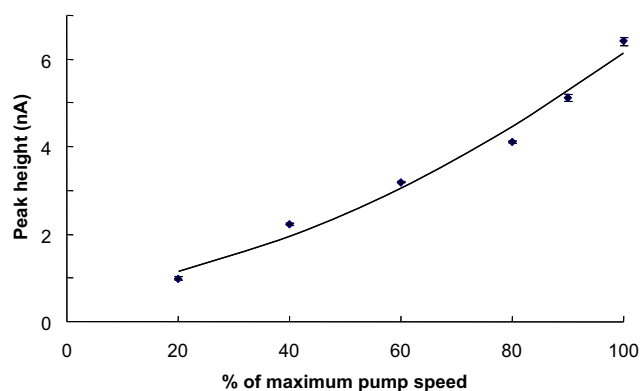


Figure 3. Influence of flow rate on chemiluminescence signal. Conditions: H_2O_2 $10^{-2}\text{mol}\cdot\text{dm}^{-3}$; luminol $10^{-2}\text{mol}\cdot\text{dm}^{-3}$ in $\text{Na}_2\text{CO}_3\text{-NaHCO}_3$ buffer solution (pH=10.7); Cr(III) $10\text{ng}\cdot\text{cm}^{-3}$; PMT voltage 900V. Each point represents a mean of six determinations ($\pm\text{SD}$).

Flow rate: The influence of the flow rate of the reagents was examined in the range of $1.24\text{-}4.61\text{cm}^3\cdot\text{min}^{-1}$, which corresponded with the percentage of maximum speed of peristaltic pump ranging from 20 to 100%. The amount of Cr(III) entering the system per time unit was proportional to the flow rate, so that the faster flow rate gave greater sensitivity. Decrease of the photocurrent with the drop in the flow rate could be an effect of the increasing the residence time of the chemiluminescence reagent in the system. We observed that the increase in peak height was almost proportional to the flow rate (Figure 3). However, at the higher flow rate we observed worse repeatability of chemiluminescence signal. The RSD increased from the level of about 0.1% (for the flow rate from 1.24 to $3.85\text{cm}^3\cdot\text{min}^{-1}$) to about 1.6% (for the flow rate higher than $3.85\text{cm}^3\cdot\text{min}^{-1}$). As an optimum we have chosen the flow rate of $3.85\text{cm}^3\cdot\text{min}^{-1}$ (80% of a maximum pump speed).

Sample volume: Influence of sample volume on the chemiluminescence intensity is shown on Figure 4. The maximum emission intensity was attained with the volume of 0.15cm^3 , which corresponded to the working time of valve V2 (position "1") (Figure 1) equal 2.4s (4 times 0.6s, Figure 2 part b) at a speed of the pump $3.85\text{cm}^3\cdot\text{min}^{-1}$. Hence, this volume was used for the sample analysis.

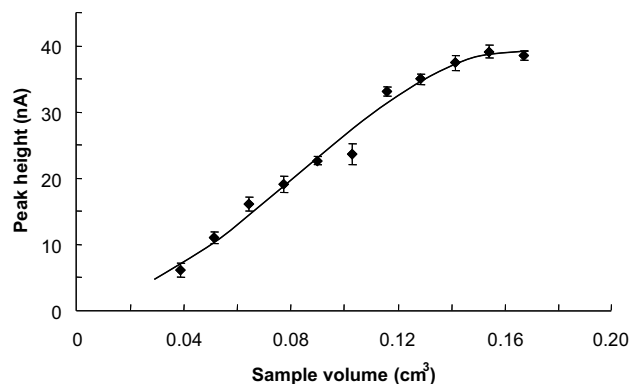


Figure 4. Influence of sample volume on chemiluminescence signal. Conditions: H_2O_2 $10^{-2}\text{mol}\cdot\text{dm}^{-3}$; luminol $10^{-2}\text{mol}\cdot\text{dm}^{-3}$ in $\text{Na}_2\text{CO}_3\text{-NaHCO}_3$ buffer solution (pH=10.7); Cr(III) $20\text{ng}\cdot\text{cm}^{-3}$; PMT voltage 900V. Each point represents a mean of 6 determinations ($\pm\text{SD}$).

Length of mixing coils: To obtain efficient introduction of Cr(III) sample to the mixture luminol/ H_2O_2 , the influence of the coil situated between valve V2 and flow cell FC (Figure 1) was examined. With the increase in length of the coil from 15 to 70cm, the chemiluminescence signal decreased about 40%. The length of mixing coil changed the dispersion of the sample: longer coil caused larger dispersion and lower intensity of chemiluminescence. Another explanation could be that the higher reactor caused higher sample dispersion, but also increased the residence time, i.e. the maximum of CL emission could occur before the sample zone reached the measurement cell. Therefore, we concluded that the coil must be long enough to mix all reagents, and, at the same time, as short as possible to let obtaining narrow and high peaks.

To obtain the mixture of the reagents and sample it was possible to use only electromagnetic valve V2 (without mixing coil). The solvents were introduced alternatively and the time of aspiration of appropriate solutions (and as a result their volumes) was optimized. This method for sample and reagent mixing is named "binary sampling" (Catala-Icardo et al. 2002). The best results were obtained by using the combination (0.6, 0.6) (Figure 2, part b). That means, that at first the sample was aspirated for 0.6s, then for the next 0.6s the reagents were aspirated, and then, in the next cycle, again the sample and the reagents. To obtain the optimized volume of the sample of about 0.15cm^3 , four aspirations of the sample for 0.6s each were necessary (it means that the total time of the sample aspiration was 2.4s). According to our investigations, this method of mixing allowed the increase of the chemiluminescence signals by about 35%. Moreover, the registered signals were more reproducible. Therefore, multicommutation was selected as the best way of mixing the sample with the solution of the reagents. Distance from the valve V2 to the detector was as short as possible.

In order to improve the efficiency of mixing the luminol with the hydrogen peroxide, the coil of the length 39cm was used. Additionally, multicommutation mixing with the application of valve V1 was used. The final, optimized program of valve V1 and V2 is shown on Figure 2, part b. The valve V2 began to work 8s later than the valve V1. This time was long enough for the mixture luminol/H₂O₂ to cover the distance between valve V1 and V2. The time of the whole measuring cycle was 40 seconds.

Effect of chemical variables

Luminol concentration: The influence of the concentration of the luminol on the CL reaction was examined in the range from 10⁻⁴ to 10⁻²mol·dm⁻³. With the increase of the luminol concentration, both the CL signals and the background noises increased. The signal to noise ratio (S/N) reached its maximum for the luminol concentration of 10⁻³mol·dm⁻³. Therefore, this concentration was selected as the optimum one.

H₂O₂ concentration: The solution of the luminol reacts with hydrogen peroxide to produce light emission. The influence of the H₂O₂ concentration was examined in the range of 10⁻³ to 10⁻¹mol·dm⁻³. The highest S/N ratio and the best repeatability were obtained for 10⁻²mol·dm⁻³. Thus, this concentration was used in subsequent experiments.

EDTA concentration: The luminol-H₂O₂ reaction is catalysed by Cr(III) and other metal ions. The addition of EDTA greatly reduced the luminescence because of the formation of the metal-EDTA complexes. The Cr(III)-EDTA complex is thermodynamically stable but kinetically slow to form at a room temperature. All other metal ions that catalyse the luminol-H₂O₂ reaction rapidly form complexes with EDTA (Escobar et al. 1993, 1998; Seitz et al. 1972). Therefore, the addition of EDTA to mask foreign ions improved the selectivity of determination of Cr(III).

The effect of the EDTA was investigated in two different ways: (a) EDTA was added to sample solution as a masking

agent, (b) EDTA was added to carrier (H₂O₂) in order to decrease and stabilise the baseline.

The influence of the EDTA concentration in the sample solution was examined in the range from 10⁻⁷ to 10⁻³mol·dm⁻³. We observed that chemiluminescence signal decreased drastically with the increasing EDTA concentration over 10⁻⁵mol·dm⁻³. Hence, 10⁻⁵mol·dm⁻³ concentration of the EDTA in the sample was chosen for masking metal ions.

According to the recommendation of Escobar et al. (1998), we checked the influence of 10⁻⁴mol·dm⁻³ EDTA on the baseline noises. It has been found that the baseline was more stable and the noises decreased even by 70%. Therefore, we always prepared the sample solution and the carrier with the addition of the EDTA.

Analytical characteristics

Selectivity: To confirm the selectivity of the method, the effect of several heavy metal ions at the concentrations 10, 25 and 100ng·cm⁻³ was studied (Table 1). Selectivity depends on the Cr(III) and on the other metal concentration. It was possible to obtain satisfying level of selectivity only at the concentration of 100ng·cm⁻³ (about 105%). The addition of masking agent to the samples caused worse sensitivities (Moliner-Martinez et al. 2003). This was especially essential at the low concentrations of Cr(III). For example, when the concentration of Cr(III) and other ions were at 10ng·cm⁻³, the selectivity decreased to about 60-70%. This problem should be solved before the method of the Cr(III) analysis in environmental samples at a very low concentrations is applied.

Another problem was the presence of organic matter in the samples. In the literature data we have found the information that organic matter can cause distinct interferences in the determination of Cr(III). Therefore, a total destruction of organic matter in the sample is fundamental before measuring, especially when biological samples, such as the hair, blood, serum or urine, are analysed (Escobar et al. 1998).

Table 1. Influence of EDTA addition on selectivity in Cr(III) determination. Conditions: H₂O₂ 10⁻²mol·dm⁻³; luminol 10⁻³mol·dm⁻³; EDTA in sample 10⁻⁵mol·dm⁻³; pH=10.2. Instrumental optimum conditions.

Ion	Photocurrent intensity* (nA)		Selectivity (%)
	Cr ³⁺ + EDTA	Cr ³⁺ + EDTA + ion(s)	
Metal concentration: 10ng·cm⁻³; PMT voltage: 900V			
Ni ²⁺	13.97±0.20	9.904±0.300	71
Zn ²⁺		8.914±0.300	64
Cu ²⁺		8.713±0.200	62
Metal concentration: 25ng·cm⁻³; PMT voltage: 700V			
Ni ²⁺	164.9±4.9	119.3±2.6	72
Zn ²⁺		155.0±4.2	94
Cu ²⁺		145.2±3.6	88
Metal concentration: 100ng·cm⁻³; PMT voltage: 500V			
mixture of Ni ²⁺ , Zn ²⁺ , Cu ²⁺	49.93±1.20	49.29±1.40	105

* Mean of 5 determinations±standard deviation.

Calibration graph: Chemiluminescence signal of Cr(III) ions was not linearly proportional to the concentration. Numerous authors construct calibration graph as a plot of log peak height in the function of log Cr(III) concentration (Escobar et al. 1993; Moliner-Martinez et al. 2003; Tortajada-Genaro et al. 2001). In other words, a straight line was obtained for double logarithmic calibration plot:

$$\log I = \log a + b \log C_{Cr}$$

where: I – chemiluminescence intensity,
a, b – constants,
 C_{Cr} – Cr(III) concentration.

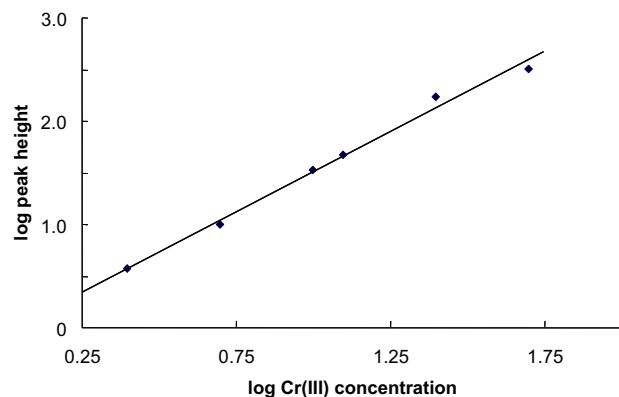


Figure 5. Calibration graph for Cr(III) determination. Optimal conditions: H_2O_2 $10^{-2}mol \cdot dm^{-3}$ in EDTA ($10^{-4}mol \cdot dm^{-3}$); luminol $10^{-2}mol \cdot dm^{-3}$ in $Na_2CO_3 \cdot NaHCO_3$ buffer solution (pH=10.7); Cr(III) from 2.5 to $50ng \cdot cm^{-3}$; flow rate $3.85cm^3 \cdot min^{-1}$; sample volume $0.15cm^3$; PMT voltage 700V. Each point represents a mean of 10 determinations.

Figure 5 presents the double logarithmic calibration graph. It was constructed according to the data obtained in optimised multicommutation experimental conditions for the concentration of Cr(III) ions from 2.5 to $50ng \cdot cm^{-3}$ (PMT voltage 700V). This calibration was prepared by proper dilution of standard Cr(III) ions with deionised water. The regression equation was $\log I = -0.041 + 1.552 \log C_{Cr}$ ($r^2=0.9916$). The range of calibration graph was in agreement with the results obtained by Seitz et al. (1972), where the linearity is limited by

precipitation of $Cr(OH)_3$ from the solution of high Cr(III) content. The upper limit of the Cr(III) concentration according to our investigation was $50ng \cdot cm^{-3}$ (Seitz et al. (1972): $10^{-6}mol \cdot dm^{-3}$, what means about $52ng \cdot cm^{-3}$).

The theoretical detection limit defined as the analyte concentration, giving a signal equal to the blank signal plus three standard deviations of the blank, was $0.15ng \cdot cm^{-3}$. The relative standard deviation (RSD) was 1.80% for 10 determinations of $20ng \cdot cm^{-3}$ Cr(III). The sampling rate was $90 samples \cdot h^{-1}$. Consumption of reagents (cm^3 per peak) was: luminol 0.26; H_2O_2 2.16; sample 0.15. Table 2 compares the characteristics of elaborated multicommutation method to the FIA method applied in determination of chromium(III) ions described by Escobar et al. (1993, 1995, 1998).

It needs emphasizing the main advantages of the multicommutation: high precision, low reagents consumption and large sample throughput. The main advantage of the multicommutation was the possibility of full automation of the analysis. Human factor influencing repeatability has been reduced to the minimum. As a result the precision of the method increased in comparison to the FIA method (regarded as being a very precise one). In the FIA mode all the reagents (H_2O_2 and the luminol) are introduced to the system in a continuous way. Multicommutation enables significant reduction of sample and reagent consumption (especially of the expensive luminol).

Applications: The proposed method was applied to the determination of chromium(III) ions in tap water. The amount of Cr^{3+} was determined using elaborated double logarithmic calibration graph. Signals for calibration graph were registered at the same conditions and during the same day. Recovery of the method was calculated from the equation:

$$R(\%) = \frac{(X+S_i) - (X)}{S} \cdot 100\%$$

where: S – added, known amount of Cr^{3+} ions
(2, 4 or $6ng \cdot cm^{-3}$),

$X+S_i$ – found concentration of Cr^{3+} in the sample
(with addition of known amount of Cr^{3+}),

X – found concentration of Cr^{3+} in the sample.

Table 2. Analytical parameters of elaborated multicommutation method and flow injection analysis (FIA). Both methods apply chemiluminescence detection based on luminol reaction. FIA parameters follow Escobar et al. (1993, 1995, 1998).

Analytical parameter	Multicommutation	FIA
Detection limit ($ng \cdot cm^{-3}$)	0.15	0.01 or 0.02
Linear range ($ng \cdot cm^{-3}$)	2.50 – 50.00	0.01 – 6.00
Precision (RSD%)	1.8	2.5 or 6.0
Sampling rate ($samples \cdot h^{-1}$)	90	70
Sample volume (cm^3)	0.15	0.40
Reagent consumption (cm^3 per peak)		
H_2O_2	2.16	1.75
Luminol	0.26	2.08

Table 3. Results of determination of Cr(III) in the samples of tap water (see text for details).

Cr ³⁺ added S (ng·cm ⁻³)	Peak height* (nA)	Cr ³⁺ found X + S _i (ng·cm ⁻³)	Cr ³⁺ found X (ng·cm ⁻³)	Recovery (%)
Tap water	2.50±0.05	–	1.92	–
2	6.58±0.07	3.71	–	83
4	12.70±0.10	5.47	–	89
6	20.20±0.10	7.36	–	91

* Mean of 10 determinations±standard deviation,
– not measurable.

Presented results (Table 3) indicated that the accuracy of the method was satisfactory (recovery 83-91%) considering that the analysis was performed at a trace level (ng·cm⁻³, i.e. ppb level).

CONCLUSION

A multicommutation flow method can be adapted to the determination of the Cr(III). The results of this study indicated that the multicommutation analysis enabled achievement of the high and repeatable analytical signal, which in turn allowed improving of the precision of the method. Proposed method was faster and cheaper than the FIA. Recovery experiment performed with the tap water indicated that the multicommutation with chemiluminescence detection can be used for the monitoring of chromium(III) ions in the environmental samples. Nevertheless, we suggest that in the case of the samples with more complicated and rich matrix (biological samples), the problem with poor selectivity should be solved by adequate sample preparation, e.g. sample mineralization and then, maybe, appropriate separation.

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