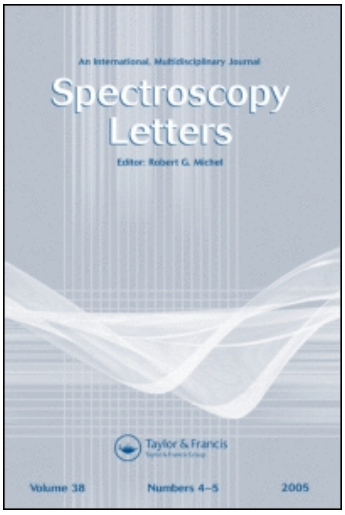

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Spectrophotometric Determination of Thiocyanate in Human Saliva Employing Micropumping Multicommutation Flow System

Joilton J. Silva Júnior[a]; Marcelo A. Farias[a]; Valdinete L. Silva[b]; Maria C. B. S. M. Montenegro[c]; Alberto N. Araújo[c]; André F. Lavorante[d]; Ana Paula S. Paim[a]
[a] Universidade Federal de Pernambuco, Departamento de Química Fundamental, Cidade Universitária, Recife-PE, Brazil [b] Universidade Federal de Pernambuco, Departamento de Engenharia Química, Cidade Universitária, Recife-PE, Brazil [c] Universidade do Porto, Faculdade de Farmácia, REQUIMTE, Departamento de Química Física, Porto, Portugal [d] Universidade Federal Rural de Pernambuco, Departamento de Química, Recife-PE, Brazil

Online publication date: 05 April 2010

To cite this Article Silva Júnior, Joilton J. , Farias, Marcelo A. , Silva, Valdinete L. , Montenegro, Maria C. B. S. M. , Araújo, Alberto N. , Lavorante, André F. and Paim, Ana Paula S.(2010) 'Spectrophotometric Determination of Thiocyanate in Human Saliva Employing Micropumping Multicommutation Flow System', Spectroscopy Letters, 43: 3, 213 — 219
To link to this Article: DOI: 10.1080/00387010903287094
URL: http://dx.doi.org/10.1080/00387010903287094

Spectroscopy Letters, 43:213–219, 2010

ISSN: 0038-7010 print/1532-2289 online
DOI: 10.1080/00387010903287094

Taylor & Francis
Taylor & Francis Group

Spectrophotometric Determination of Thiocyanate in Human Saliva Employing Micropumping Multicommutation Flow System

Joilton J. Silva Júnior[1], Marcelo A. Farias[1], Valdinete L. Silva[2], Maria C. B. S. M. Montenegro[3], Alberto N. Araújo[3], André F. Lavorante[4], and Ana Paula S. Paim[1]

[1]Universidade Federal de Pernambuco, Departamento de Química Fundamental, Cidade Universitária, Recife-PE, Brazil
[2]Universidade Federal de Pernambuco, Departamento de Engenharia Química, Cidade Universitária, Recife-PE, Brazil
[3]Universidade do Porto, Faculdade de Farmácia, REQUIMTE, Departamento de Química Física, Porto, Portugal
[4]Universidade Federal Rural de Pernambuco, Departamento de Química, Recife-PE, Brazil

ABSTRACT A procedure based on a multicommutated approach for the determination of thiocyanate (SCN^-) in human saliva is described. The method was based on the reaction of Fe(III) with SCN^-, producing a red complex that was monitored at 480 nm. Under optimum experimental conditions, a linear response ranging from 0.5 to 10.0 m mol L^{-1} SCN^- ($R = 0.9991$), a relative standard deviation of 1.0% ($n = 10$) for a sample 5 m mol L^{-1} SCN^-, a detection limit (3σ criterion) of 50 μ mol L^{-1}, a sampling rate of 60 determinations per hour, reagent consumption of 0.02 mg Fe(III) per determination, and a waste generation of 1.6 mL per determination were achieved.

KEYWORDS human saliva, multicommutation flow analysis, multipumping, spectrophotometry, thiocyanate

INTRODUCTION

Human saliva is a complex biofluid excreted by the salivary glands. Saliva composition includes air, water, proteins, peptides, amino acids, hormones, electrolytes, lipids, and other substances.[1,2] Salivary composition, however, can be influenced by physiological factors as well as individual personal features and diet.[3]

Thiocyanate (SCN^-) is generated in the human organism during the digestion of food, mainly vegetables of the cabbage family,[4] as well as when drugs are ingested for the treatment of thyroid and hypertension,[5] in addition to generation by metabolic products of substances present in tobacco smoke that contain cyanide.

SCN^- is a metabolite of cyanide detoxification with similar toxicity that has received attention from researchers in different fields such as medicine, food chemistry, and environmental sciences.[2] Saliva with a high level of SCN^- is an indicator of cyanide poisoning, which could be caused by chronic human exposure to hydrogen cyanide. SCN^- toxicity effects include anorexia, nausea, fatigue, disorientation, and psychosis.[6,7] For these

Received 3 April 2009; accepted 25 July 2009.

Address correspondence to Ana Paula S. Paim, Universidade Federal de Pernambuco, Departamento de Química Fundamental, Av. Prof. Luiz Freire, s/n. Cidade Universitária, 50740-550 Recife-PE, Brazil. E-mail: anaspaim@ufpe.br

reasons, plasmatic thiocyanate concentration should be monitored in order to ensure that its concentration does not exceed 0.1 mg mL^{-1}.

SCN^- is considered a biomarker of environmental tobacco smoke exposure. The level of thiocyanate in saliva, urine, and blood serum[8,9] is thus considered a good way to distinguish smokers from nonsmokers and its determination is useful to evaluate smoking behavior.[10] Whereas the saliva of nonsmokers presents SCN^- concentrations in the range between 0.5 to 2.0 m mol L^{-1},[3,9] in smokers, a concentration of about 6.0 m mol L^{-1} has been found.[2]

Various methods to determine thiocyanate in wastewaters,[11,12] synthetic samples,[13] biological samples,[5–8,10,11,14–16] and food[16] have been proposed. Spectrophotometers,[5–7,9,12,13,17–19] potentiometry with ion-selective electrodes,[11,14] and fluorimetry[15] have all been employed as detection techniques. Separation processes such as ionic chromatography have also been used.[8,16] Though some methods present high sensitivity,[11,14,15,17] they are subject to interferences caused by concomitant species.[11,14,15] Others are fast and simple but present low sensitivity.[5,7,12,13,18,19] Furthermore, some proposed methods required complex instruments[6,16,18,19] or the use of hazardous reagents.[8,9,17]

As was pointed out above, SCN^- determination is very important for human health therefore, the availability of fast and reliable analytical procedures using inexpensive equipment and a simple setup process is recommended. It is possible to meet these requirements by employing a multicommuted flow injection analysis process (MCFIA),[20,21] which allows facilities to achieve reduction of reagent consumption and waste generation.

In the analytical procedures employing an MCFIA approach, the peristaltic pump is by far the device most often used to propel a solution, although the solenoid micropump has also been used for solution pumping.[22] The advantages of this device are its smaller dimension and its propelling and commuting ability, which permit the replacement of the peristaltic pump and solenoid valves in a flow system based on multicommuted flow injection analysis.[23–31] A flow system that uses solenoid micropumps makes different flow rates possible by varying the on/off switching frequency. The electronic hardware required to drive the solenoid micropump is similar to that usually employed in the multicommutation process.[21] The inherent features of the MCFIA, such as versatility, low reagent consumption, and robustness, are also enhanced using a solenoid micropump to propel sample and reagent solutions.[28]

In this work, we intend to describe the development of a multicommuted flow injection procedure for the spectrophotometric determination of thiocyanate in human saliva employing solenoid micropumps to propel reagent solutions. The photometric method selected is based on the reaction of thiocyanate with Fe(III) in an aqueous medium, which presents radiation absorption at 480 nm.[32,33]

EXPERIMENTAL

Reagents and Solutions

All chemicals were of analytical reagent grade. Purified water (electric conductivity lower than 0.1 μS cm^{-1}) was used throughout.

A 9.0 m mol L^{-1} Fe(III) solution was prepared by dissolving 0.5018 g of metallic iron in the mixture of 10.0 mL of HNO_3 concentrated (Merck, Germany) plus 30.0 mL of HCl concentrated (Merck, Germany). After dissolution, water was added to complete the volume to 1000 mL.

A 0.015 mol L^{-1} thiocyanate stock solution was prepared by dissolving 0.360 g KSCN (Merck, Germany) in 250 mL of water. Working solutions containing 0.5–10.0 m mol L^{-1} thiocyanate were prepared by appropriate dilution of the stock solution with water.

In the interference studies, solutions using the following salts were prepared: potassium nitrate (KNO_3), sodium sulphate (Na_2SO_4), sodium chlorate (NaCl), calcium chlorate ($CaCl_2$), magnesium chlorate ($MgCl_2$), and sodium citrate ($Na_3C_6H_5O_7$).

Sample Preparation

Human saliva samples were collected from smokers and nonsmokers in glass tubes. Prior to collection, donors washed their mouths first with a 5 g L^{-1} citric acid solution (saliva stimulator) and then three times with water.[18] The saliva samples were centrifuged at 2500 rpm for 10 min. Afterwards, samples were diluted with an appropriate volume of water and analyzed.

Apparatus

The flow system comprised three solenoid micropumps (8 μL per stroke; Bio-Chem 090SP, Boonton,

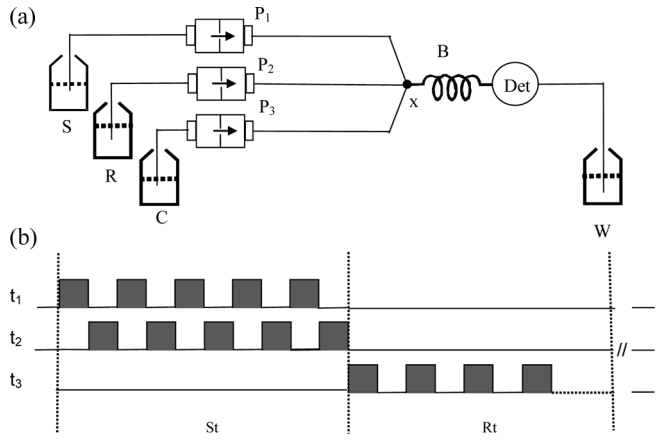

FIGURE 1 **Flow diagram of the multicommuted flow system. (a) Diagram of the flow system. P_1, P_2, and P_3: solenoid micropumps; S: sample; R: reagent solution; C: carrier solution, B: reaction coil, 0.8 mm i.d., 100 cm; x: confluence; Det: detector, 480 nm; W: waste. (b) Switch time course of micropumps, respectively, t_1, t_2, and t_3. St: Sampling step; Rt: Signal reading step.**

NJ); a microcomputer furnished with an electronic interface card PCL711S (American Advantech Co.); a 700 S Femto spectrophotometer (Brazil) equipped with a flow cell, 10-mm optical path and 80-μL inner volume (Hellma, Germany); a homemade electronic interface[34] to drive the solenoid micropumps; and a four-way acrylic connector. The reaction coil and flow lines were made of polytetrafluoroethylene (PTFE) tubing, 0.8 mm i.d. Control and data acquisition from the micropumps were performed by the microcomputer running software written in Quick BASIC 4.5.

Flow Procedure

Figure 1a shows the flow diagram of the system. All pumps are switched off, and thus no solution is flowing through the system. When the software was running, the microcomputer sent control signals through the PCL711 interface card to switch the solenoid micropumps on/off, in the pattern, as depicted in Fig. 1b.

As we can see, micropumps P_1 and P_2 were switched on/off alternately five times, thus inserting a string comprising five slugs of sample solution in tandem with five slugs of reagent solution into the reaction coil (B). Afterwards, the micropump P_3 was switched on/off several times to establish a stream of the carrier solution to displace the sample zone toward the photometer (Det). The mixing of solutions between slugs proceeded while the sample zone was displaced through the reaction coil B, thus causing a reaction that produced the compound that was monitored at 480 nm.

Upon execution of the software program, the microcomputer requested the control parameters shown in Table 1. Afterwards, the analytical procedure was carried out as depicted in the pump switching pattern. The time interval used to maintain switched on/off micropumps P_1 and P_2 was settled at 0.1 s; thus, the switching frequency was 5 Hz. Each micropump delivered 8 μL per stroke during the sampling step (St, Fig. 1), thus inserting equal volumes (40 μL) of sample and reagent solution into the reaction coil. Afterwards, the micropump P_3 was switched on/off (Rt, Fig. 1) several times to maintain the frequency of 5 Hz, propelling the carrier solution through the reaction coil (B) to displace the sample zone toward the detector at a flow rate of $40\,\mu L\,s^{-1}$. The signal generated by the spectrophotometer (Det) was read by the microcomputer through a serial interface and stored as an ASCII file to allow further treatment. While the analytical process was in process, a signal plot was displayed on the computer screen as a time function to allow its visualization in real time.

RESULTS AND DISCUSSION

Effect of Reagent Concentration

The effect of reagent concentration on the response range was investigated using a set of reference solutions with concentrations ranging from 0.5 up to $10.0\,m\,mol\,L^{-1}$ SCN^-, yielding the results shown in Fig. 2. As we can see, the monitored signal increased

TABLE 1 **Solenoid Micropumps Switching Course for Thiocyanate Determination**

Step	Description	P1	P2	P3	Pulses	Cycles
1	Sample or SCN^- standard introduction	On/off[a]	Off	Off	1	5
2	Reagent solution Fe^{3+} introduction	Off	On/off	Off	1	
3	Transport to the detection cell and system washing	Off	Off	On/off	200	—

[a]On/off indicates a 0.1 s/0.1 s pulse of the solenoid micropump.

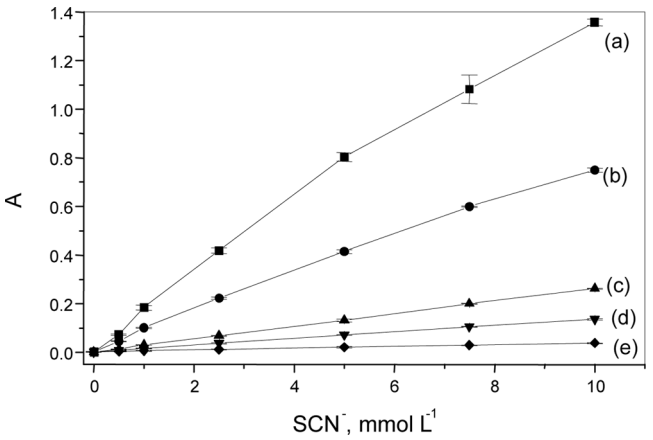

FIGURE 2 **Effects of the reagent solution concentration on the linearity of the analytical curve. Experimental conditions: 5 cycles, ratio 1:1 solution volume: (a) 1.8×10^{-2}; (b) 9.0×10^{-3}; (c) 3.6×10^{-3}; (d) 1.8×10^{-3}; (e) 7.2×10^{-4} mol L^{-1} Fe^{3+}. The results were obtained using a 5 m mol L^{-1} SCN^- standard solution.**

with iron (III) concentration. A linear response (R = 0.999) was observed, however, when the reagent concentration was 9.0 m mol L^{-1} Fe^{3+} or less. Given these results, a reagent concentration of 9.0 m mol L^{-1} Fe^{3+} was chosen to carry out further experiments. In these assays, each sampling cycle comprised one on/off switching step of micropumps P1 and P_2. Each micropump delivered 8 μL per stroke, and because five sampling cycles were carried out, the volume of the sample zone was 80 μL.

Effect of Reagent Slug Volume

Because reagent volume might affect the sensitivity of the procedure, a set of experiments was carried out in order to ascertain optimum conditions. Slugs of the reagent with volumes of 8, 16, and 24 μL were inserted and the volume of the sample slug was maintained at 8 μL. These assays were carried out using the set of SCN^- standard solutions described above, carried out by switching on/off micropump P_2 one, two, and three times to comprise each sampling cycle, and the micropump P_1 was switched on/off once. When iron (III) solution slugs of volumes 16 and 24 μL were used, the analytical signals showed an increase of 70% and 112%, respectively, when compared with the use of a slug volume of 8 μL. However, no linear relationship with SCN^- concentration was observed. Better results (R = 0.999) were achieved when the volume of the reagent slug solution was 8 μL. These results agree with those presented in the previous section, where the reagent concentration was increased, linearity was lessened, and the magnitude of the signal increased. This effect could be expected considering that the concentration of $[Fe^{3+}(SCN^-)_n]^{3-n}$ depends on the concentration of SCN^-.[35]

Effect of Reaction Coil Length

The results discussed above were obtained using a 100-cm reaction coil. Because the dimension of the reaction coil may affect sensitivity, precision, and sampling rate, a set of assays was performed varying the length of the coil from 10 to 200 cm. The best results considering signal magnitude, linearity, and precision were obtained when a reaction coil with a length of 100 cm was used. A slightly decreased signal magnitude was observed for reaction coils that were longer. We could suppose that the decrease in signal was caused by a dispersion effect, which increased with the length of the reaction coil.

Effect of Sampling Cycles

The results commented on before were obtained by performing five sampling cycles, resulting in an 80-μL sample zone volume (40 μL of sample solution and 40 μL reagent solution). Considering that volume of the sample zone might affect the signal magnitude, additional experiments were carried out employing six and eight sampling cycles. With the increased number of sampling cycles, the signals increased 28% and 28.5%, respectively. Even so, five sampling cycles was considered to be sufficiently sensitive to process samples of human saliva as well as saving the reagent, reducing waste, and improving the sampling rate.

Interfering Effect

The main concomitant ions present in human saliva that could cause interference are Ca^{2+}, Mg^{2+},

TABLE 2 **Tolerance Concentration Ratio (Interferent/SCN^-) of Several Species in the Determination of SCN^- (5.0 m mol L^{-1} SCN^-)**

Foreign species	Tolerance limit[a]
Ca^{2+}, Mg^{2+}, NO_3^-	100[b]
SO_4^{2-}, Cl^-, Na^+, citric acid	10

[a]Data are interferent/analyte concentration ratios, in m mol L^{-1}.
[b]Maximum tested ratio.

TABLE 3 Determination of SCN^- Concentration in Human Saliva as Determined by the Proposed Method and by Standard Addition Method[a,b]

Samples[c]	Proposed method	Standard addition method	Recovery[d] (%)
1	1.1 ± 0.1	1.2 ± 0.3	104 ± 7
2	0.8 ± 0.1	0.7 ± 0.2	92 ± 2
3	0.6 ± 0.1	0.8 ± 0.1	93 ± 6
4	0.5 ± 0.1	0.6 ± 0.1	103 ± 1
5	0.8 ± 0.1	0.9 ± 0.1	107 ± 8
6	0.5 ± 0.1	0.6 ± 0.1	101 ± 1
7	2.0 ± 0.1	1.7 ± 0.1	97 ± 9
8	1.5 ± 0.1	1.5 ± 0.2	104 ± 4
9	1.9 ± 0.2	1.6 ± 0.2	90 ± 5
10	1.8 ± 0.1	1.7 ± 0.1	94 ± 4
11	2.1 ± 0.1	2.0 ± 0.2	92 ± 3

[a]Results expressed in $m\,mol\,L^{-1}$.
[b]$n = 3$.
[c]Samples 1–6: nonsmokers; 7–11: smokers.
[d]After spiking $1.0\,m\,mol\,L^{-1}$.

Na^+, NO_3^-, SO_4^{2-}, Cl^-, and citrates. The studies were performed to verify whether the presence of these ions in the sample caused interference (matrix effect). Analyzing the results displayed in Table 2, the least tolerance observed occurred for SO_4^{2-}, Cl^-, Na^+, and citric acid. The complex $[Fe^{3+}(SCN^-)_n]^{3-n}$, which only has a pKf of 3.02, is less stable than $Fe_2(SO_4)_3$ (pKf = 4.18). A tolerance limit up to 10 times the concentration of SCN^- (interferent/SCN^-) would not cause interference, however.

An acceptable tolerance for the assays was considered to be an absorbance variation of ±2% as a result of the interfering ion. Table 2 demonstrates results using this criterion. These results are higher than those normally found in this sample type.[1,2]

Results Comparison and System Performance

Once the optimal operational conditions had been established, a set of human saliva samples was analyzed in order to test the usefulness of the proposed procedure. The standard addition method was also used to test the accuracy of the assessment samples, yielding the results shown in Table 3. As we can see, the recoveries obtained with the addition of $1.0\,m\,mol\,L^{-1}$ SCN^- to each of the samples are within the range of 90–107%, which is considered acceptable for this type of sample. The theoretical t value (2.23) for a 95% probability level and 10 degrees of freedom is less than the t calculated (1.70), indicating that both procedures provide statistically comparable results.

The calibration graph is linear over the range $0.5–10.0\,m\,mol\,L^{-1}$ with a detection limit of $50\,\mu\,mol\,L^{-1}$. A sampling rate of 60 determinations per hour and a relative standard deviation of 1.0% ($n = 10$) for a typical solution containing $5.0\,m\,mol\,L^{-1}$ SCN^- were also achieved.

The main parameters usually employed to evaluate the performance of the analytical procedures are summarized in Table 4. As we can see, the performance of the proposed procedure compares very well with results recorded in other published papers, mainly considering those of Gumus et al.[17] and Themelis and Tzanavaras,[18] which were applied to determine thiocyanate in human saliva. The linear response range of the proposed procedure is broad enough to determine SCN^- concentration in human saliva.[3,9] Analyzing the values related to sample and reagent consumption as well as waste generation we observed that the comparison is very favorable to the proposed procedure.

TABLE 4 Analytical Performance Comparison of the Different Procedures for Thiocyanate Determination

Parameters	Proposed procedure	[11]	[12]	[17]	[18]
Linear range ($mg\,L^{-1}$)	29–580	2.0–150.0	0–5.0	0–4.0	20.0–800
Correlation coefficient (R)	0.9991	0.9996	0.9998	0.9998	0.9980
Relative standard deviation (%)	1.0	1.2	1.8	<1.0	1.0
Limit of detection ($mg\,L^{-1}$)	2.9	1.1	0.08	0.007	5.0
Sample volume (μL)*	40	50	70	180	200
Reagent consumption (mg)*	0.02	4.9	14.7	897	3.1
Waste generation (mL)**	1.6	1.5	3.1	4.4	10
Throughput (hr^{-1})	60	24	10	60	—

The labels * and ** indicate that the values are related to consumption and waste generation per determination, respectively.

CONCLUSIONS

The micropumping multicommutation flow system was successfully applied to determine thiocyanate in human saliva samples and to distinguish smokers from nonsmokers. The statistics from which this conclusion was drawn are considered to be at a 95% confidence level with a theoretical t value of 2.26 for 9 degrees of freedom, when the value of t is 70.69. This indicates a significant difference between the concentration of SCN^- found in the saliva of smokers and that in nonsmokers.

The proposed system is simple, fast, and easy to operate, with the additional advantage of being robust and having low reagent consumption, resulting in low waste generation. The solenoid micropump could be viewed as a commuting device and in this sense a set of pumps can be easily assembled to comprise the hardware for the handling of reagent solutions controlled by software.

ACKNOWLEDGMENTS

The authors are grateful to CAPES (Brazil)/ GRICES (Portugal), FACEPE, and CNPq for financial support and to those individuals who provided the saliva samples to make this investigation feasible.

REFERENCES

1. Lac, G. Saliva assays in clinical and research biology. *Pathol. Biol.* **2001**, *49*, 660–667.
2. Valdés, M. G.; Díaz-García, M. E. Determination of thiocyanate within physiological fluids and environmental samples: current practice and future trends. *Crit. Rev. Anal. Chem.* **2004**, *34*, 9–23.
3. Gay, J.-Y.; Fovet, Y.; Adib-Yadzi, M. About a synthetic saliva for in vitro studies. *Talanta* **2001**, *53*, 1103–1115.
4. Butts, W. C.; Kuehneman, M.; Widdowson, G. M. Automated method for determining serum thiocyanate, to distinguish smokers from nonsmokers. *Clin. Chem.* **1974**, *20*, 1344–1348.
5. Pinillos, S. C.; Vicente, I. S.; Bemal, J. G.; Asensio, J. S. Determination of thiocyanate by carbonyl sulphide (OCS) generation and gas-phase molecular absorption spectrometry. *Anal. Chim. Acta.* **1996**, *318*, 377–383.
6. García, M. S.; Ortuño, J. A.; Sánchez-Pedreño, C.; Albero, M. I.; Fernández, M. J. Flow-through bulk optode for spectrophotometric determination of thiocyanate and its application to water and saliva analysis. *Sensors* **2006**, *6*, 1224–1233.
7. Tsuge, K.; Kataoka, M.; Seto, Y. Cyanide and thiocyanate levels in blood and saliva of healthy adult volunteers. *J. Health Sci.* **2000**, *46*, 343–350.
8. Torano, J. S.; van Kan, H. Simultaneous determination of the tobacco smoke uptake parameters nicotine, cotinine and thiocyanate in urine, saliva and hair, using gas chromatography-mass spectrometry for characterisation of smoking status of recently exposed subjects. *Analyst* **2003**, *128*, 838–843.
9. Bashammakh, A. S.; Bahaffi, S. O.; Al-Sibaai, A. A.; Al-Wael, H. O.; El-Shihawi, M. S. Extractive liquid-liquid spectrophotometric procedure for the determination of thiocyanate ions employing the ion pair reagent amiloride monohydrochloride. *Anal. Chim. Acta.* **2007**, *592*, 16–23.
10. Demkowska, I.; Polkowska, Z.; Namiesnik, J. Application of ion chromatography for the determination of inorganic ions, especially thiocyanates in human saliva samples as biomarkers of environmental tobacco smoke exposure. *J. Chromatogr. B* **2008**, *875*, 419–426.
11. Ardakani, M. M.; Jamshidpour, M.; Naeimi, H.; Moradi, L. Thiocyanate ion-selective PVC membrane electrode based on N,N′-ethylene-bis(4-methylsalicylidineiminato)nickel(II). *Anal. Sci.* **2006**, *22*, 1221–1226.
12. van Staden, J.; Botha, A. Spectrophotometric determination of thiocyanate by sequential injection analysis. *Anal. Chim. Acta.* **2000**, *403*, 279–286.
13. Sweileh, J. A. Determination of cyanide and thiocyanate by a spectrophotometric flow-injection method. *Anal. Chim. Acta.* **1989**, *220*, 65–74.
14. Hassan, S. S. M.; Mohmoud, W. H.; Elgazwy, A. S. S. H.; Badawy, N. M. A novel potentiometric membrane sensor based on aryl palladium complex for selective determination of thiocyanate in the saliva and urine of cigarette smokers. *Electroanalysis* **2006**, *18*, 2070–2078.
15. Gong, B.; Gong, G. Fluorimetric melthod for the determination of thiocyanate with 2′,7′-dichlorofluorescein and iodine. *Anal. Chim. Acta.* **1999**, *394*, 171–175.
16. Bjergegaard, C.; Moller, P.; Sorensen, H. Determination of thiocyanate, iodide, nitrate and nitrite in biological samples by micellar electrokinetic capillary chromatography. *J. Chromatogr. A* **1995**, *717*, 409–414.
17. Gumus, G.; Demirata, B.; Apak, R. Simultaneous spectrophotometric determination of cyanide and thiocyanate after separation on a melamine-formaldehyde resin. *Talanta* **2000**, *53*, 305–315.
18. Themelis, D. G.; Tzanavaras, P. D. Solvent extraction flow-injection manifold for the simultaneous spectrophotometric determination of free cyanide and thiocyanate íons based upon on-line masking of cyanides by formaldehyde. *Anal. Chim. Acta.* **2002**, *452*, 295–302.
19. Chamjangali, M. A.; Bagherian, G.; Salek-Gilani, N. Determination of trace amounts of thiocyanate by a new kinetic procedure based on an induction period. *Spectrochim. Acta.* **2007**, *67*, 1252–1256.
20. Reis, B. F.; Giné, M. F.; Zagatto, E. A. G.; Lima, J. L. F. C.; Lapa, R. Multicommutation in flow analysis. Part 1. Binary sampling: concepts, instrumentation and spectrophotometric determination of iron in plant digests. *Anal. Chim. Acta.* **1994**, *293*, 129–138.
21. Lavorante, A. F.; Feres, M. A.; Reis B. F. Multicommutation in flow analysis: A versatile tool for the development of the automatic analytical procedure focused on the reduction of reagent consumption. *Spectros. Lett.* **2006**, *39*, 631–650.
22. Lapa, R. A. S.; Lima, J. L. F. C.; Reis, B. F.; Santos, J. L. M.; Zagatto, E. A. G. Multi-pumping in flow analysis: Concepts, instrumentation, potentialities. *Anal. Chim. Acta.* **2002**, *466*, 125–132.
23. Carneiro, J. M. T.; Zagatto, E. A. G.; Santos, J. L. M.; Lima, J. L. F. C. Spectrophotometric determination of phytic acid in plant extracts using a multi-pumping flow system. *Anal. Chim. Acta.* **2002**, *474*, 161–166.
24. Lima, J. L. F. C.; Sá, S. M. O.; Santos, J. L. M.; Zagatto, E. A. G. Multi-pumping flow system for the spectrophotometric determination of dipyrone in pharmaceutical preparations. *J. Pharmaceut. Biomed. Anal.* **2003**, *32*, 1011–1017.
25. Dias, A. C. B.; Santos, J. L. M.; Lima, J. L. F. C.; Zagatto, E. A. G. Multi-pumping flow system for spectrophotometric determination of bromhexine. *Anal. Chim. Acta.* **2003**, *499*, 107–113.
26. Prior, J. A. V.; Santos, J. L. M.; Lima, J. L. F. C. Sampling strategies exploiting multi-pumping flow systems. *Anal. Bioanal. Chem.* **2003**, *375*, 1234–1239.
27. Lavorante, A. F.; Morales-Rubio, A.; de la Guardia, M.; Reis, B. F. Micro-pumping flow system for spectrophotometric determination of anionic surfactants in water. *Anal. Bioanal. Chem.* **2005**, *381*, 1305–1309.

28. Rocha, F. R. P.; Cordova, C. M. I.; Melchert, W. R. A multi-purpose flow system based on multi-commutation. *Spectros. Lett.* **2006**, *39*, 651–668.
29. Pons, C.; Tóth, I. V.; Rangel, A. O. S. S.; Forteza, R.; Cerdà, V. Multi-pumping flow system for the determination of dissolved orthophosphate and dissolved organic phosphorus in wastewater samples. *Anal. Chim. Acta.* **2006**, *572*, 148–154.
30. Ródenas-Torralba, E.; Morales-Rubio, A.; Lavorante, A. F.; Reis, B. F.; de la Guardia, M. Micropumping multicommutation turbidimetric analysis of waters. *Talanta* **2007**, *73*, 742–747.
31. Lima, J. L. F. C.; Santos, J. L. M.; Dias, A. C. B.; Ribeiro, M. F. T.; Zagatto, E. A. G. Multi-pumping flow systems: an automation tool. *Talanta* **2004**, *64*, 1091–1098.
32. Paim, A. P. S.; Kronka, E. A. M.; Reis, B. F.; Korn, M. Determinação espectrofotométrica de ácido ascórbico em fármacos empregando amostragem binária em fluxo. *Quím. Nova.* **1998**, *21*, 47–50.
33. Recalde-Ruiz, D. L.; Andrés-García, E.; Díaz-García, M. E. Development of flow analytical systems for monitoring thiocyanate biodegradation in waste waters. *Anal. Lett.* **2000**, *33*, 1603–1614.
34. Ródenas-Torralba, E.; Rocha, F. R. P.; Reis, B. F.; Morales-Rubio, A.; de la Guardia, M. Evaluation of a multicommuted flow system for photometric environmental measurements. *J. Automat. Meth. Manag. Chem*. **2006**, *2006*, 1–9.
35. Mendham, J.; Denney, R. C.; Barnes, J. D.; Thomas, M. J. K. *Vogel—Análise Química Quantitativa*, 6th ed.; LTC: Rio de Janeiro, 2002.