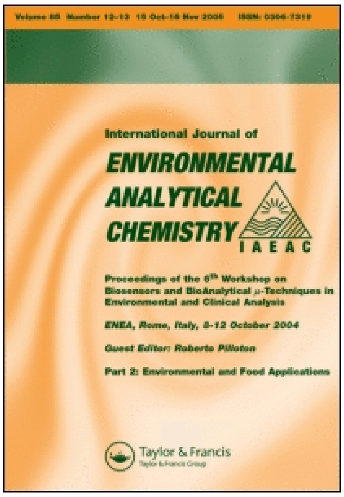

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A multi-pumping flow system for chemiluminescent determination of ammonium in natural waters

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A multi-pumping flow system for chemiluminescent determination of ammonium in natural waters

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A multi-pumping flow system with improved mixing conditions is proposed for chemiluminescent determination of ammonium in natural waters. The system includes a gas-diffusion unit, a Y-connector made of sintered glass particles (100–200 mesh), and a chemiluminometric detector. System configuration, flow rates, reagent concentrations, pH, and reactor lengths were optimized. The analytical curve is linear from 0.3 to 5.0 mg L^{-1} N-NH_4, and the detection limit is estimated as 0.02 mg L^{-1} at the 99.7% confidence level. The system handles about 50 samples per hour, requires 3.1 µg of active chlorine and 0.11 mg of luminol per determination, and yields precise results (RSD < 1.2%, $n = 10$) in agreement with those obtained by the spectrophotometric indophenol blue method.

Keywords: Flow analysis; Ammonium; Multi-pumping; Chemiluminescence; Water analysis

1. Introduction

Chemiluminescent (CL) analytical techniques are receiving increasing attention due to their inherent characteristics of sensitivity, detection limit, dynamical concentration range, etc. As an external radiation source is not required, source instability, stray light, and background radiation are not usually limiting factors.

CL is amenable to be implemented in a flow system, and the related analyser usually presents high versatility, simplicity, and ruggedness [1, 2]. CL reactions are usually fast, and this is often a limiting factor in the flow system design. Rapid mixing of the solutions yielding the light emission, preferably close to the detector, may circumvent this limitation [2]. This is better accomplished in a multi-pumping flow system (MPFS) [3–5], which allows efficient and reproducible solution mixing.

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MPFS comprises several discretely actuated solenoid micro-pumps acting as insertion ports, propelling devices and mixing elements. Their exploitation allows the design of fully mechanized, highly versatile systems characterized by improved mixing conditions. Moreover, reagents are not continuously inserted but timely added to the sample zone, thus reducing consumption of reagents. Another relevant feature of MPFS is the pulsed flow, which leads to a chaotic solution movement thus providing rapid mixing of sample/reagents under low dispersion conditions [4]. This aspect makes MPFS attractive for chemiluminescent determinations.

Mixing conditions can also be improved through the efficient addition of merging streams, and the geometry of the connectors plays a relevant role in this context [6].

Gas diffusion units have often been coupled to flow analysers for monitoring volatile species [7, 8]. Ammonia collection by an acceptor stream can be accomplished in an MPFS with a gas-diffusion unit, and the approach can minimize matrix effects and provide a sensitivity enhancement by in-line analyte concentration.

Ammonium concentration in natural waters is related to the nutrient availability. High concentrations may increase the biological activity, leading often to toxicity as well as to alterations in taste and smell [9]. In water plant treatments, ammonium monitoring is required for decision making related to quality of the water to be consumed.

CL procedures for ammonium determination usually involve analyte oxidation by hypobromite [10] or hypochlorite [11] and further radiation emission resulting from the luminol oxidation by the remaining oxidizing agent. This latter reaction has been exploited in relation to immobilized or electro-generated chemiluminescent reagents [12], core wave-guides for sensitivity enhancement [13] or proposal of a portable analyser [3].

The aim of this work was then to develop an improved MPFS exploiting gas-diffusion for CL determination of ammonium in natural waters. The method relies on the oxidation of luminol by hypochlorite under acidic conditions. To guarantee enhanced sample/reagent mixing, lower sample dispersion and higher sampling rates, a specially designed Y-connector made of sintered glass beads was used.

2. Experimental

2.1 *Samples, standards, and reagents*

The solutions were prepared with freshly deionized water (conductivity $<0.1\,\mu S\,cm^{-1}$) and chemicals of analytical-grade quality.

The samples were collected into 100-mL polyethylene bottles, filtered through $0.45\,\mu m$ cellulose membrane filters, and analysed on the same day [14].

The weekly prepared stock standard solution ($300\,mg\,L^{-1}$ N-NH_4 in $0.01\,mol\,L^{-1}$ HCl) was based on NH_4Cl. Working standards within the 0.30–6.00 $mg\,L^{-1}$ N-NH_4 range were prepared daily by water dilutions of the stock solution.

The $5.0 \times 10^{-3}\,mol\,L^{-1}$ luminol solution was prepared by dissolving 88.8 mg in 100 mL of a $0.3\,mol\,L^{-1}$ carbonate buffer solution based on Na_2CO_3, with the pH adjusted to 10.5. The reagent was stored in an amber flask and maintained in a

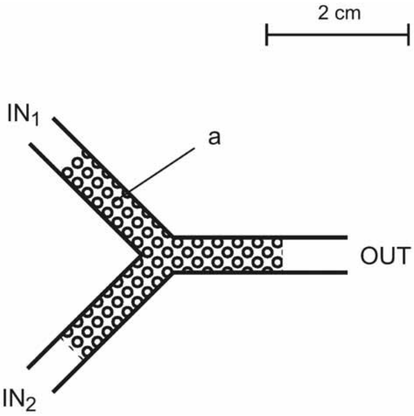

Figure 1. Y-connector. (a) sintered glass. Manifold tubing is attached by means of external Tygon gloves.

refrigerator when not in use. The daily prepared 0.1% (w/v) active chlorine solution was based on an iodometrically standardized [15] 5.0% (w/v) $NaClO_2$ solution.

2.2 *Apparatus*

The solenoid micro-pumps (Bio-Chem Valve Inc., Boston, MA) with a stroke volume of 8 or 25 µL were actuated through the ULN 2003 integrated circuit. The gas-diffusion unit and the chemiluminometric detector (40-µL flow cell) were similar to those already described [7, 16]. Coiled reactors and transmission lines were made from 0.8 mm i.d. PTFE tubing. The Y-connector (figure 1) was made by adding glass particles (100–200 mesh) inside a conventional open tubular glass connector, and sintering inside a muffle furnace.

A microcomputer with a model PCL-818L Advantech interface card performed data acquisition and treatment, as well as system control. Software was developed in Quickbasic 4.5.

2.3 *Flow diagram*

An MPFS without the gas-diffusion unit (figure 2a) was initially designed to allow the evaluation of the main involved parameters. It comprised four micro-pumps for propelling and directing the luminol, hypochlorite, buffer, and sample solutions.

The analytical cycle started by pumping the carrier stream (P_4 pump on), thus washing the analytical path. Thereafter, the P_4 pump was switched off, and the other pumps on, in order to add the hypochlorite and the sample solutions (×confluence) as well as the luminol reagent (y confluence). Consequently, ammonium underwent oxidation to chloramines, and luminol reacted with the remaining hypochlorite inside the B_1 reactor. After a pre-selected time interval, the P_1, P_2, and P_3 pumps were switched off, and the P_4 pump was turned on, directing the sample zone towards detection. Passage of the sample through the detector resulted in a transient decrease in

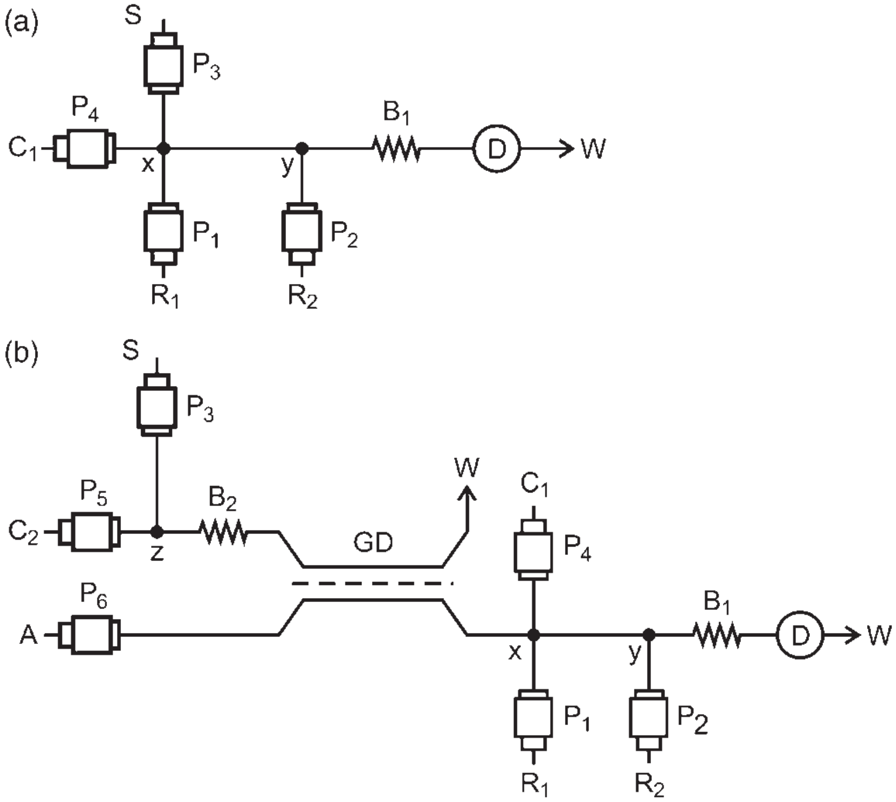

Figure 2. Flow diagrams of the MPFS without (a) and with (b) the gas diffusion unit. S: sample; C_1: carbonate buffer ($0.3\,\mathrm{mol\,L^{-1}}$, pH 10.5); C_2: $0.1\,\mathrm{mol\,L^{-1}}$ NaOH; R_1: 0.0025% hypochlorite; R_2: $5.0 \times 10^{-3}\,\mathrm{mol\,L^{-1}}$ luminol; A: $0.005\,\mathrm{mol\,L^{-1}}$ HCl; P_i: micro-pumps; B_i: reactors; GD: gas-diffusion unit (PTFE membrane in traced line); *x* and *z*: conventional connectors; *y*: glass sintered Y-connector; *x–y*: transmission line; *D*: detector (420 nm); *W*: collecting flask for waste disposal.

emitted light intensity, which was quantified at 420 nm and recorded as a peak. The peak height was then inversely proportional to the analyte content in the sample.

The analytical signal (ΔI) was calculated as the difference in heights of the peaks recorded in the absence (I_A) or presence (I_B) of ammonium.

For selectivity enhancement, an analogous system incorporating a gas-diffusion unit (figure 2b) was designed. P_1–P_4 pumps were operated as mentioned above, whereas the P_5 and P_6 pumps were used for propelling the sodium hydroxide and hydrochloric acid solutions, respectively.

In this system, the P_3 and P_5 pumps were switched on, directing the selected volumes of sample and alkaline solutions towards the z confluence. Ammonium was converted to ammonia inside the following B_2 reaction coil, which diffused through the PTFE membrane towards the acidic collector stream. This stream was stopped during the gas-diffusion step, so the analyte was concentrated in line, leading to a sensitivity enhancement. Next, the P_1, P_2, and P_6 pumps were switched on, in order to add the luminol, hypochlorite, and acceptor solutions, respectively. These micro-pumps were thereafter turned off, and the sample zone was directed towards detection through actuation of just the P_4 pump. Further sample processing and analytical signal attainment were analogous as described above.

2.4 *Procedure*

The main parameters related to the 2A system, such as sample volume, flow rates, and reagent concentrations, were optimized by the univariate method, aimed at a compromise involving sensitivity, repeatability, sampling rate, and reagent consumptions. To this end, the working standard solutions were processed in triplicate after every modification of the system. Parameters more closely related to the gas-diffusion step were investigated by adding the gas-diffusion chamber to the manifold (figure 2b). The main figures of merit of the resulting MPFS were evaluated, and the system was applied to the water analyses.

For accuracy assessment, different samples (tap water, river water, and wastewater) were also analysed by the phenate method, which involved formation of the indophenol blue compound, and spectrophotometric measurement at 640 nm.

3. Results and discussion

3.1 *Evaluation of the system without gas diffusion*

Influence of the sample volume, defined by pump stroke volume and number of pulses [17], was investigated for the largest pump (25 µL stroke) by varying the number of pulses between 3 and 15, meaning sample volumes within 75 and 375 µL. The system depicted in figure 2(a) was used and, for each variation in number of pulses, the hypochlorite and luminol reagent volumes were varied accordingly.

The effects of hypochlorite and luminol concentrations were investigated within the 0.001–0.025% (w/v) and $1.0\text{–}7.5 \times 10^{-3}\,\mathrm{mol\,L^{-1}}$ ranges, and the best sensitivity was attained with 0.0025% (w/v) and $5.0 \times 10^{-3}\,\mathrm{mol\,L^{-1}}$, respectively.

Regarding sample volume, increasing the number of pulses from three to eight increased the ΔI value in an asymptotic manner. Beyond eight pulses, no further improvement in sensitivity was noted, and sampling rate deteriorated. As a compromise between sensitivity and sampling rate, five pulses were selected.

The influence of reactor length was investigated between 5 and 40 cm. A ΔI increase with the reactor length was always noted, the effect being more pronounced for shorter reactors (<15 cm). The tendency was also noted for longer reactors, yet with less intensity, and is a consequence of the relatively slow ammonium oxidation by hypochlorite. This parameter was then selected as 15 cm. The use of such a short reactor was possible in view of the improved mixing conditions determined by the establishment of a pulsed flow and by the use of the special connector. In fact, reproducible measurements (RSD < 2%) were always attained. It should be emphasized that a 30% decrease in analytical signal was noted by replacing the Y-connector by a conventional one.

Total flow rate is also a relevant parameter because if the flow rate is too low or too high, the maximum intensity of the emitted radiation would occur before the sample reached the detector or even after leaving it. As the pump strokes were fixed, the influence of total flow rate was investigated by varying the pulse frequency between 100 and 300 min^{-1}. In this way, changing the time intervals corresponding to the stop periods determined the variations in total flow rate. No significant variations in ΔI were noted, thus confirming that diffusion is not relevant when the sample is stopped. This aspect corroborated earlier findings. It should be noted that, when the stopped-flow approach was conceived [18], diffusion during the stop period was considered as irrelevant. For longer reactor lengths, higher flow rates should be selected, thereby increasing the consumption of reagents. The pulse frequency was then selected as 200 min^{-1}, meaning a 5.0 mL min^{-1} total flow rate.

Another relevant parameter was the pH of the reaction medium, and the best results were obtained for a pH of 10.5. This value is in close adherence with earlier work [19]. It should be noted that no analytical signals were obtained for $\text{pH} < 9.5$ or $\text{pH} > 12$, thus emphasizing the need for proper system buffering.

In order to verify the feasibility of applying the system without gas diffusion (figure 2a) to water analyses, the influence of potential interfering ions was investigated. To this end, different solutions with 0.00 or 1.68 mg L^{-1} N-NH_4 containing also one of the tested ions were prepared and introduced into the system. Na^+, SO_4^{2-}, K^+, Cl^-, and NO_3^- did not interfere, even under a 1000-fold molar ratio, as peak height deviations were lower than 5%. Br^-, Ca^{2+}, and Mg^{2+} were tolerated up to 100-fold molar ratios; Zn^{2+}, Al^{3+} and phosphate were tolerated up to 10-fold. At similar molar ratios, Mn^{2+}, Ni^{2+}, Cu^{2+}, Fe^{2+}, and Fe^{3+} yielded a significant chemiluminescence quenching. In view of these pronounced interfering effects, it was decided to exploit in-line gas diffusion, and the MPFS modified in order to include the gas-diffusion chamber.

3.2 *Evaluation of the system with gas diffusion*

The influence of the acceptor stream concentration was investigated in order to provide guidelines for designing the MPFS (figure 2b) to be applied to water analysis. The concentration of the acceptor stream was varied between 0.001 and 0.05 mol L^{-1} HCl, and the highest ΔI values were noted for 0.005 mol L^{-1}. The decrease in analytical signal observed for higher acidities is a consequence of the lower pH of the reaction medium. The concentration of the acceptor stream was then selected as 0.005 mol L^{-1} HCl; with this relatively low acidity, it was not necessary to modify the previously defined reagent concentrations.

The influence of alkalinity of the donor stream was studied by varying the NaOH concentration (up to 1.0 mol L^{-1}) of the sample carrier stream (C_2; figure 2b). Only slight differences in analytical signals (<5%) were noted within 0.1 and 1.0 mol L^{-1} OH^-, so the lower value was selected. The influence of flow rate of the donor stream was investigated within 0.82 and 5.0 mL min^{-1} by varying the pulse frequency related to the S and C_2 streams. The best sensitivity was verified for a pulse frequency of 252 min^{-1} that corresponds to 0.82 mL min^{-1}. This value reflects individual 0.20 and 0.62 mL min^{-1} flow rates for the sample and C_2 solutions provided by the 8- and 25-µL pumps.

In order to enhance sensitivity, the possibility of stopping the acceptor stream during the gas-diffusion step was investigated. The alkaline sample zone was continuously flowing during the gas-diffusion process, and thus the in-line analyte concentration was attained. The number of pulses related to S and C_2 streams (figure 2b) was varied during the stop period, and the best results were noted for 20 pulses. Increasing this manifold parameter beyond this value increased the ΔI value but also led to a decrease in sampling rate.

The length of the B_2 reactor was also investigated and selected as 20 cm. Despite this short reactor, measurements were precise (RSD < 1.2%), thus emphasizing the good mixing conditions provided by the pulsed flow.

3.3 *Application*

The proposed system (figure 2b) involves the addition of five pulses of the sample solution (125 µL), simultaneously with the luminol and hypochlorite reagents, a stop period of 0.8 min, a 5.0 mL min^{-1} total flow rate, and a 15-cm B_1 reactor. This was applied to the analysis of surface and waste waters.

The linearity of the analytical curve was verified between 0.3 and 5.0 mg L^{-1} N-NH_4, and the detection limit was estimated [20] as 0.02 mg L^{-1}. These figures allowed the determination of ammonium at the concentration levels defined by the European legislation for water for human consumption [21]. A typical analytical curve is described as:

$$\Delta I = -17.03C + 3.36 \quad r = 0.997, \quad n = 7,$$

where ΔI is the difference in blank and analytical signals (mV), and C is the analyte concentration (mg L^{-1} N-NH_4).

The sampling rate is about 50 h^{-1}, meaning 3.1 µg of active chlorine (or 62 µg of sodium hypoclorite) and 0.11 mg luminol per determination. This low consumption of reagents is a consequence of the exploitation of pulsed flows and use of the Y-connector. Under improved mixing conditions, shorter reactors are required, thus lessening the sample and reagent amounts. Baseline drift is not noted after 8-h operation periods, thus emphasizing the system ruggedness.

The proposed system yields precise results (RSD usually <1.2%) in agreement with the reference procedure (table 1). This agreement is described by $D = 1.0067$, $R - 0.0441$, where D and R are the results obtained by the developed and the reference methods, respectively, with a correlation coefficient of 0.9997. A student t-test further

Table 1. Ammonium concentrations (mg L^{-1}) in natural waters as determined by the proposed and reference [14] methods.

Sample	Proposed	Reference	Relative deviation (%)
Tap water 1	nd	0.19	–
Tap water 2	nd	0.17	–
River water 1	0.65 ± 0.01	0.64	+1.56
River water 2	0.77 ± 0.01	0.75	+2.67
River water 3	1.08 ± 0.08	1.02	+5.90
River water 4	0.95 ± 0.01	0.92	+3.26
Waste water 1	1.16 ± 0.05	1.21	−4.13
Waste water 2	18.5 ± 0.83	18.1	+2.18
Waste water 3	18.1 ± 1.2	18.4	−1.65
Waste water 4	30.6 ± 0.28	30.9	−0.98

nd: not detectable.

confirmed this agreement: the estimated *t*-value (0.842) was lower than the tabulated value (2.37) for a confidence level of 95% ($n = 7$).

To evaluate the interfering effect of several ionic species commonly present in water samples, a 1.68 mg L^{-1} ammonium standard was run by the developed procedure (with gas diffusion) in the presence of increasing amounts of interferent. Results confirmed the good selectivity provided by the utilization of a gas-diffusion unit reported in previous works [7, 8].

4. Conclusions

The proposed system for CL determination of ammonium in natural waters exploits the simplicity, versatility, and ease of operation of MPFS by coupling a gas-diffusion cell that enables implementation of an in-line separation/concentration step. A stopped acceptor stream collected the analyte. The pulsed nature of the flowing stream generated by solenoid micro-pumps, combined with the inclusion of a special sinterized glass Y-connector for merging streams, provided improved mixing conditions, and so the results were precise, and reagent consumptions were low.

The proposed system is a good alternative for ammonium determination in water analysis, as it does not require the use of large amounts of hazardous or toxic reagents. This follows the general tendency towards a clean chemistry. The present procedure uses very known and easily available chemicals. The favourable characteristics of CL-based procedures are enhanced, as the MPFS-CL hyphenation led to a simple, fully automated and rugged system able to handle 50 samples per hour in spite of the involved in-line analyte separation/concentration step.

The use of solenoid micro-pumps as solution drivers, propelling devices, and commuters, as well as the use of a simple luminometer resulted in a low-cost and highly versatile analyser. This latter aspect became evident when gas diffusion was implemented: system reconfiguration was easily accomplished.

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References

[1] A.C. Calokerinos, N.T. Deftereos, W.R.G. Baeyens. *J. Pharm. Biomed. Anal.*, **13**, 1063 (1995).
[2] C.K. Pires, B.F. Reis, C.X. Galhardo, P.B. Martelli. *Anal. Lett.*, **36**, 3011 (2003).
[3] F.R.P. Rocha, E. Rodenas-Torralba, B.F. Reis, A. Morales-Rubio, M. de la Guardia. *Talanta*, **67**, 673 (2005).
[4] R.A.S. Lapa, J.L.F.C. Lima, B.F. Reis, J.L.M. Santos, E.A.G. Zagatto. *Anal. Chim. Acta*, **466**, 125 (2002).
[5] J.L.F.C. Lima, J.L.M. Santos, A.C.B. Dias, M.F.T. Ribeiro, E.A.G. Zagatto. *Talanta*, **64**, 1091 (2004).
[6] G.D. Clark, J.M. Hungerford, G.D. Christian. *Anal. Chem.*, **61**, 973 (1989).
[7] M.T. Oms, A. Cerda, A. Cladera, V. Cerdá, R. Forteza. *Anal. Chim. Acta*, **318**, 251 (1996).

[8] M.D. Luque De Castro, M. Valcarcel. *Anal. Chim. Acta*, **261**, 425 (1992).
[9] American Public Health Association, American Water Works Association and Water Pollution Control Federation. *Standard Methods for the Examination of Water and Wastewater*, p. 496, American Public Health Association, New York (1975).
[10] X. Hu, N. Takenaka, S. Takasuna, M. Kitano, H. Bandow, Y. Maeda, M. Hattori. *Anal. Chem.*, **65**, 3489 (1993).
[11] P.R. Kraus, S.R. Crouch. *Anal. Lett.*, **20**, 183 (1987).
[12] W. Qin, Z. Zhang, B. Li, Y. Peng. *Talanta*, **48**, 225 (1999).
[13] J. Li, P.K. Dasgupta. *Anal. Chim. Acta*, **398**, 33 (1999).
[14] L.S. Clesceri, A.E. Greenberg, A.D. Eaton. *Standard Methods for the Examination of Water and Wastewater*, 20th Edn, p. 108, American Public Health Association, Washington, DC (1998).
[15] *Farmacopeia Portuguesa VII* (Official Edition), Vol. I, Infarmed, Lisbon (2002).
[16] M. Miro, J.M. Estela, V. Cerda. *Anal. Chim. Acta*, **541**, 57 (2005).
[17] A.F. Lavorante, A. Morales-Rubio, M. de la Guardia, B.F. Reis. *Anal. Bioanal. Chem.*, **381**, 1305 (2005).
[18] J. Ruzicka, E.H. Hansen. *Anal. Chim. Acta*, **106**, 207 (1979).
[19] C.K. Pires, P.B. Martelli, B.F. Reis, J.L.F.C. Lima, M.L.M.F.S. Saraiva. *J. Autom. Meth. Manag. Chem.*, **25**, 109 (2003).
[20] IUPAC—Analytical Methods Committee. *Analyst*, **112**, 199 (1987).
[21] *European Directive 98/83/CE.*