Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 60 (2003) 369–376
Talanta
www.elsevier.com/locate/talanta

Determination of tyrosine through a FIA-direct chemiluminescence procedure

M.C. Sanfeliu Alonso [a], L. Lahuerta Zamora [a], J. Martínez Calatayud [b,*]

[a] Departamento de Química, Bioquímica y Biología Molecular, Universidad Cardenal Herrera – C.E.U., 46113 Moncada, Valencia, Spain
[b] Departamento de Química Analítica, Facultad de Ciencias Químicas, Universidad de Valencia, 46100 Burjassot, Valencia, Spain

Received 16 July 2002; received in revised form 23 October 2002; accepted 25 October 2002

Abstract

A new FI-direct chemiluminescence method is proposed for the determination of tyrosine, based on the oxidation of the amino acid by $K_3Fe(CN)_6$ in potassium hydroxide medium, at room temperature and enhanced by the presence of β-cyclodextrin and formic acid. The dynamic range was linear over the range 1.0–10.0 mg l^{-1}. A large study of the influence of foreign compounds was performed, including amino acids; and, the method showed high selectivity. The reproducibility between days resulted in a rsd (in slope%) of 4.8 and the repeatability with a rsd ($n = 50$, 10.0 mg l^{-1}) of 3.1%, the LOD ($s/n = 3$) was 50 μg l^{-1} and sample throughput 98 h^{-1}.

Keywords: Tyrosine; Chemiluminescence; Continuous-flow; Pharmaceuticals

1. Introduction

Tyrosine is a nonessential amino acid. Its biosynthesis in mammals occurs by hydroxylation of phenylalanine. Tyrosine is precursor of compounds with biological interest as melanin, dopa and dopamine, triiodo- and tetraiodothyronine, norepinephrine and epinephrine. Failures in metabolic pathway of tyrosine results in some inherited diseases like phenilketonuria, tyrosinemia and tyrosinosis.

There are a number of analytical methods dealing with tyrosine determination, namely, spectrophotometric [1–6], fluorimetric [7–9] and electroanalytical [10–13]. Other procedures deal with the prior chromatographic separation and further determination of the amino acid [14–16]. There are four papers dealing with the indirect chemiluminometric detection of tyrosine on the basis of inhibition or enhancement of other chemiluminescent systems, like 4-iodophenol-luminol-hydrogen peroxyde [17], luminol-hydrogen peroxyde [18] and tris(bipyridyl)ruthenium(III) [19,20]. Searching for analytical methods (source: Analytical Abstract, from 1980 to September 2001), authors only found one paper [21] dealing with the direct-chemiluminometric determination of tyrosine by

* Corresponding author. Tel./fax: +34-96-354-40-62; http://www.uv.es/ ~ martinej.
E-mail addresses: lahuerta@ceu.upv.es (L. Lahuerta Zamora), jose.martinez@uv.es (J. Martínez Calatayud).

0039-9140/03/$ - see front matter
doi:10.1016/S0039-9140(03)00099-7

oxidation with potassium permanganate in sulfuric acid medium.

Although a number of papers dealing on FI-direct chemiluminescence of organic compounds [22] have been published, this continues as a growing area in analytical chemistry [23–26] and experimental efforts (screening procedures) are made to find direct chemiluminescent behavior of different kinds of substances. This goal is mainly focused to the analytical advantages that could be obtained by coupling the FI methodology with the direct chemiluminescence. Analytical advantages like low detection limits, wide linear dynamic ranges, high speed of response and reproducible mixing of sample and reagents near the detector.

Screening of more than 150 organic compounds (most of them pharmaceuticals), was performed by examining their reaction with four common oxidants (permanganate, cerium(IV), hexacyanoferrate(III) and hydrogen peroxyde) in different media. Tyrosine was found to be chemiluminescent when it reacts in alkaline medium with potassium hexacyanoferrate(III). One of the earliest analytical applications [27] has been employed so far in the direct-chemiluminescence determination of only a very limited number of substances [28–30], besides its use in chemiluminescence reactions involving the oxidation of luminol [31–33].

As far as the authors know, this is the first attempt to determine the tyrosine by a FIA-direct chemiluminescence procedure.

2. Experimental

2.1. Reagents

All solutions were prepared from analytical reagent grade materials using purified water by reverse osmosis and de-ionized water.

2.2. Materials and apparatus

The finally proposed flow injection manifold for the determination of tyrosine (Fig. 1) consisted of a peristaltic pump (P, Gilson Minipuls 3) which pumped carrier (flow-rate Q_3, 10.5 ml min^{-1} of 3.0 mol l^{-1} HCOOH and 12 mmol l^{-1} of β-cyclodextrin in 6.5 mol l^{-1} KOH) and oxidant (flow-rate Q_4, 1.5 ml min^{-1} of 3.0×10^{-2} mol l^{-1} $K_3[Fe(CN)_6]$ in water) solutions through PTFE tubes (0.8 mm i.d.). A volume (V) of 2267 μl of aqueous solution resulting from the in situ mixing of tyrosine (Q_1) and 24 mmol l^{-1} β-cyclodextrin, 6 mol l^{-1} HCOOH and 13 mol l^{-1} KOH (Q_2) solutions (injection valve from Rheodyne, Model 5041) were injected into the carrier solution. Finally, both channels (Q_3 and Q_4) merged in a T shaped piece positioned 2 cm before entering the flow cell, which consists on a flat spiral-coiled quartz tube (1.0 mm i.d., 3 cm total diameter of the flow cell, without gaps between loops).

The flow cell was backed by a mirror for maximum light collection and placed 2 mm from the photomultiplier tube (end window, type 9125B16) included in the photodetector package P30CWAD5F-29 supplied by Electron Tubes Limited (Middlesex, UK). The T-piece, flow cell and photodetector package were placed in a homemade, absolutely light-tight box. The output was fed to a computer equipped with the CT1 Counter–Timer board also supplied by Electron Tubes Limited.

The equipment was periodically tested by means of the continuous-flow manifold of the Fig. 2 and the formerly reported chemiluminescent system [30], and consisting of: Q_1, thioridazine hydrochloride, 400 mg l^{-1}; Q_2 and Q_3, sulfuric acid 1 mol l^{-1} and Q_4, Ce(IV) and ammonium nitrate 0.02 mol l^{-1} in sulfuric acid 0.5 mol l^{-1}. The obtained signal was fairly reproducible over a period of 6 months ($n = 4$, rsd = 5.6%).

3. Results and discussion

3.1. Preliminary work

The goal of the experimental work was to develop a FI procedure for the determination of tyrosine. Bearing in mind that chemiluminescence signal intensity is time-dependent, preliminary work was performed by means of a continuous-flow manifold. The time of measurement after mixing of solutions is accurately controlled by the

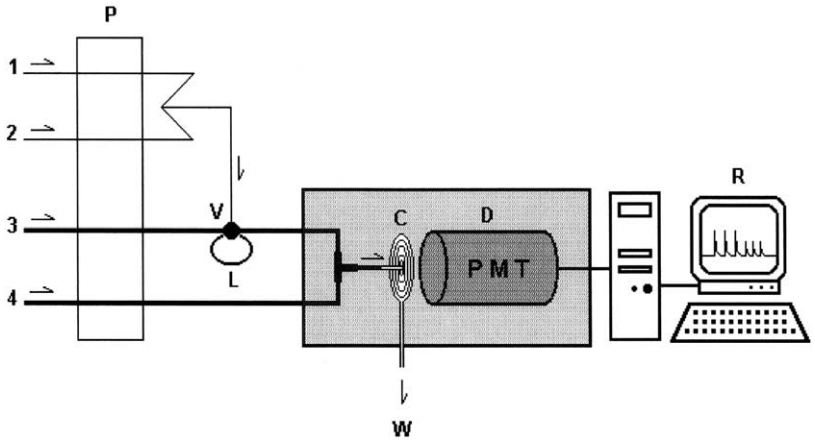

Fig. 1. Finally proposed FIA assembly for tyrosine determination (see text). Q_3, 10.5 ml min^{-1} of 3.0 mol l^{-1} HCOOH and 12 mmol l^{-1} of β-cyclodextrin in 6.5 mol l^{-1} KOH; Q_4, 1.5 ml min^{-1} of 3.0×10^{-2} mol l^{-1} $K_3[Fe(CN)_6]$ in water; V, 2267 μl of aqueous tyrosine solution (Q_1) mixed in situ with the 24 mmol l^{-1} β-cyclodextrin, 6 mol l^{-1} HCOOH and 13 mol l^{-1} KOH (Q_2) solution; PMT, photomultiplier tube; P, peristaltic pump and W, waste.

flow rates. The results obtained through this simpler approach (Fig. 2), can be easily exported to an FIA system.

The reactivity of tyrosine (100 mg l^{-1}) was tested with different oxidants (all in concentration 0.005 mol l^{-1}) in different media (H_2SO_4 1 mol l^{-1} or NaOH 1 mol l^{-1}). The analytical signal was calculated as sample output minus blank. The oxidants assayed in H_2SO_4 were, $KMnO_4$, Ce(IV), KIO_4 and $K_2S_2O_8$. In NaOH medium the tested oxidants were: H_2O_2/NaClO, *N*-bromosuccinimide and $K_3Fe(CN)_6$. Only potassium ferricyanide yielded significant chemiluminescence signal and thus it was selected as the suitable oxidant.

Then, the influence of two different alkaline hydroxides (NaOH and KOH) was tested by preparing different concentrations ranging between 0.1 and 6.0 mol l^{-1}. Alkaline solution merged with either, sample and reagent (hydroxyde flowing by Q_2 and Q_3) or only with one of them (hydroxyde flowing only by Q_2 or Q_3), before the chemiluminescence reaction at the flow cell. The merging of 4.0 mol l^{-1} of KOH with both, sample and oxidant, was selected as the combination yielding the highest signal.

The influence of the oxidant concentration was also studied by testing solutions with concentrations of $K_3[Fe(CN)_6]$ rising from 10^{-4} mol l^{-1} to 5×10^{-1} mol l^{-1} and bearing in mind the results,

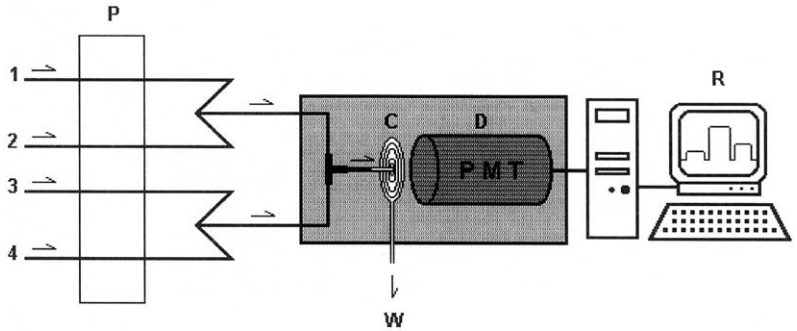

Fig. 2. Continuous-flow assembly used for the preliminary assays (see text). Q_1, aqueous maltol solution (or deionised water-blank); Q_2 and Q_3, medium; Q_4, oxidant. PMT, photomultiplier tube; P, peristaltic pump and W, waste.

a concentration of 1.5×10^{-2} mol l^{-1} was selected. The observed influence of the oxidant concentration is commonly observed in many published papers; in which high oxidant concentration results in lower chemiluminescence.

The influence of the flow rate can be critical owing to its influence over the point in the flow manifold in which the excited molecule emits the light and consequently over the signal magnitude: too low flow rates can result in maximum emission before the flow cell and too high in maximum emission after it. It was observed that flow rate should be as high as possible, which could indicate that reaction speed and emission are fast. Nevertheless, a total final flow rate of 20.4 ml min^{-1} (5.1 ml min^{-1} per channel) was selected; higher flowrates were not selected to avoid over-pressure problems in the manifold, mainly at the flow cell.

Dissolved molecular oxygen could have influence over luminescence phenomena as well as oxidation reactions which means an unpredictable influence on analytical procedures. Due to that, the influence of the concentration was also tested with two types of experimental conditions: (a) removing dissolved oxygen by passing a nitrogen stream; and, (b) increasing the oxygen content up to saturation by forcing an air stream. The chemiluminescence emission was diminished (down to 80%) for de-aerated solutions and slightly improved ($\sim$2%) when tyrosine solutions were aerated. Then, untreated solutions were selected for further work.

The influence of the temperature was tested by heating medium (after its merging with the tyrosine solution, see Fig. 2) and oxidant (after its merging with the medium solution) by immersing 2.0 m of coil in a water bath in both cases. The studied temperatures ranged from room temperature to 80 °C. The results showed a slight increase of the analytical signal of only 10% between 28 and 80 °C, which could be attributed to a faster reaction kinetic at elevated temperatures competing advantageously with a possible quenching of the chemiluminescence at elevated temperatures. However, bearing on mind the small influence of this variable and to simplify the final manifold, room temperature was selected as the most suitable.

Several compounds can act as energy transfer reagents (sensitizers) in [31] chemiluminescence reactions. Thus, different compounds, namely formic acid, rhodamine B, rhodamine 6G, quinine sulfate, acridine orange and oxine, were tested at 0.001 mol l^{-1} in the sample (Q_1) and/or water (Q_3) solution. It was found that some of them (formic acid, acridine orange and rhodamine B) increased the analytical signal while the others diminished the analytical signal down to about 84%, which could be attributed to the partial consumption of the oxidant by the sensitizer, without subsequent chemiluminescent emission. Thus, formic acid (1 mol l^{-1} flowing by Q_2 and Q_3) was selected as the most effective sensitizer, yielding an increase of 115% over the former analytical signal. Rhodamine B, rhodamine 6G, quinine sulfate, acridine orange and oxine could be sensitizers by energy transfer, since they fluoresce, but formic acid does not have a fluorogenic properties and hence it is not excited by energy transfer. Most probably, formic acid reacts with the oxidant and the energy released is transferred to the emitting molecule.

Organized media could have a marked influence on the chemiluminescence emission [32,33], and two different kinds of organizers were tested at their critical micellar concentration in the carrier or sample solution; namely, β-cyclodextrin (non-ionic organizer, may enhance chemiluminescence intensity by protection of the excited state via host–guest interactions) and hexadecylpyridinium chloride (cationic surfactant). Other commonly tested substances, like Triton X-100 (non-ionic) and sodium dodecyl sulphate (anionic) were not assayed because they precipitated in the alkaline medium. After testing different combinations and concentrations, β-cyclodextrin (flowing by Q_2 and Q_3) was selected as the best enhancer, yielding an increase of 151% over the former analytical signal.

Finally, different combinations (channels and concentrations) between the alkaline medium and the former selected sensitizer and organiser were assayed. A mixture of β-cyclodextrin 6×10^{-3} mol l^{-1}, HCOOH 2.5 mol l^{-1} and KOH 4 mol l^{-1} was selected as the best solution flowing through Q_2 and Q_3, which yielded the best compromise between magnitude of the analytical

signal and the slope of a calibration graph of tyrosine (sensitivity).

3.2. Studies in a FIA-manifold

Next, the continuous-flow manifold was changed into the FIA manifold assembly depicted in the Fig. 1; and, the formerly pre-selected variables were re-optimized on the basis of former reported studies, to adapt the chemical system to the new flow assembly.

Different combinations were tested, being always Q_1, tyrosine and Q_4, potassium ferricyanide 1.5×10^{-2} mol l^{-1} in water and studying different possibilities to introduce the KOH, HCOOH and β-cyclodextrin into the system (by Q_2 and/or Q_3), at the previously optimized concentrations. All of the experiences were performed with five different tyrosine concentrations ranging from 0.5 to 10.0 mg l^{-1}.

The best results were obtained when both, Q_2 and Q_3, were constituted simultaneously by HCOOH, β-cyclodextrin and KOH.

Concentrations of all solutions were re-optimized through an univariate procedure. The obtained results were: Q_3, 3.0 mol l^{-1} HCOOH and 12 mmol l^{-1} of β-cyclodextrin in 3 mol l^{-1} KOH; Q_4, 1.5×10^{-2} mol l^{-1} potassium ferricyanide (Fig. 3), Q_1, tyrosine and Q_2, 24 mmol l^{-1} β-cyclodextrin and 6 mol l^{-1} HCOOH in 6 mol l^{-1} KOH.

For the optimisation of FIA variables, the modified simplex multivariate method [34] was applied. The studied variables and ranges were; flow rates (Q_3 and Q_4) ranging, each channel, from 2.5 to 10.0 ml min^{-1}; sample volume (V) ranging from 152 to 1226 μl; and the reactor (L) length ranging from 0.0 to 200.0 cm. After testing 40 apices, four of them were pre-selected in order to study them comparatively. The optimum apex was selected attending to criteria of repeatability, slope of linear regression and robustness. The robustness of each apex was tested by studying the influence of small variations (five values) around each selected value through an univariated procedure. The finally selected apex was: $Q_3 = 10.5$ ml min^{-1}, $Q_4 = 1.5$ ml min^{-1}, $V = 2267.4$ μl and $L = 10.0$ cm. As it can be seen, the optima flow rates were high (which could indicate that reaction speed and emission are fast) and on the other hand, the optimum sample volume was also high, probably due to the high volume of the proposed flow cell.

With the newly adopted hydrodynamic parameters, chemical variables were then re-optimized. The refined values were: Q_3, 3.0 mol l^{-1} HCOOH and 12 mmol l^{-1} of β-cyclodextrin in 6.5 mol l^{-1} KOH (see the results of the series in Fig. 4); Q_4, 3.0×10^{-2} mol l^{-1} potassium ferricyanide; Q_1, tyrosine and Q_2, 24 mmol l^{-1} β-cyclodextrin and 6 mol l^{-1} HCOOH in 13 mol l^{-1} KOH.

At this point, it should be stressed that the high KOH employed concentrations neither supposed a considerable shortening of pump tubing lifetime nor back pressure effects over the FIA manifold.

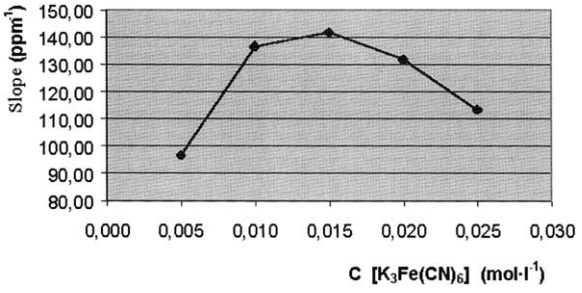

Fig. 3. Influence of the potassium ferricyanide concentration in the Q_4 stream.

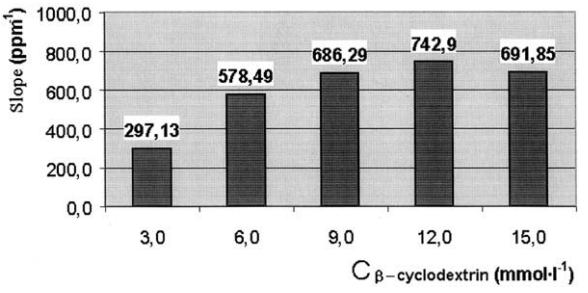

Fig. 4. Influence of the concentration of β-cyclodextrin. Figures are the slope of each tested calibration range.

4. Analytical applications

With the optimized manifold, the obtained linear dynamic range was comprised between 1.0 and 10.0 mg l^{-1}. The fitted equation was $I = 341.4C - 650.8$ ($n = 5$; $r = 0.9999$); C was the tyrosine concentration in mg l^{-1} and I was the analytical signal, namely, obtained chemiluminescence output minus blank (Q_1; water instead of tyrosine), in arbitrary units (a.u.).

The limit of detection (50 μg l^{-1}) was experimentally confirmed as the concentration yielding a signal higher than the corresponding to the blank average more than three times the standard deviation of the blank ($n = 30$). This LOD is three times smaller than the obtained through the reported batch method [21].

A between day reproducibility study was performed working each day (8 days) with freshly prepared solutions, being the tyrosine concentrations (five values) comprised in the range 1.0–10.0 mg l^{-1}. The mean ($n = 6$) slope obtained was 367.7 l mg^{-1}, with an rsd of 4.8% and mean correlation coefficient of 0.9990.

The average insertion rate for 50 peaks (10.0 mg l^{-1}) was 95 h^{-1}. The rsd thus calculated was 3.1%.

Bearing in mind the chemiluminescent reactions could be affected by the presence of different species which could be found as impurities at trace levels in commercially available reagents, to check the chemical robustness is a critical feature of the chemiluminescent systems. With this aim, each reagent (tyrosine, potassium hydroxide, formic acid, β-cyclodextrin and potassium ferricyanide) was tested from two different manufacturers, always analytical grade. Thirty-two different combinations were assayed. The system showed similar chemiluminescent emission in all cases, showing in this way its chemical robustness. The calculated rsd ($n = 32$) was 9.7% (versus 1.0 mg l^{-1} of tyrosine).

Interferences were sought among all the primary protein-forming amino acids, some of the usual components in pharmaceutical formulations containing tyrosine and their most common excipients. From 39 studied substances, only three, namely cysteine, hystidine and tryptophan, resulted in serious interference, probably because its reductive behaviour, which has been reported when they react with *N*-bromosuccinimide [35]. The interference, negative in all cases, could be attributed to the partial consumption of the oxidant by the interfering amino acid, without subsequent chemiluminescent emission. Results are shown in Table 1.

Table 1
Influence of foreign compounds versus 10.0 mg l^{-1} of tyrosine

Foreign compound	Concentration (mg l^{-1})	Er (%)
Alanine	100.0	+1.4
Arginine	100.0	−0.4
Asparagine	100.0	−0.8
Aspartic acid	100.0	+2.7
Cysteine	100.0	−99.9
Glycine	100.0	+1.6
Glutamic acid	100.0	−0.3
Glutamine	100.0	+2.2
Phenylalanine	100.0	+0.7
Hystidine	100.0	−51.3
Isoleucine	100.0	0.0
Leucine	100.0	−0.4
Lysine	100.0	+1.0
Methionine	100.0	0.0
Proline	100.0	−1.1
Serine	100.0	+1.0
Threonine	100.0	−1.6
Triptophan	100.0	−99.5
Valine	100.0	−2.9
Ascorbic acid	1.0	+6.5
Folic acid	1.0	−3.6
Biotin	100	−3.2
Cyanocobalamine	1.0	−2.4
Nicotinamide	100.0	−3.3
Sodium pantotenoate	100.0	+3.2
Pyridoxine	1.0	−3.2
Riboflavine	1.0	−8.9
Thiamin	1.0	+1.9
Sodium acetate	100.0	+0.1
Zinc acetate	100.0	+1.3
Malic acid	100.0	+5.6
Calcium chloride	100.0	+5.6
Magnesium chloride	100.0	+0.7
Potassium chloride	100.0	+2.8
Sodium chloride	100.0	+0.5
Na_2EDTA	100.0	+3.2
Sodium phosphate	100.0	+1.2
Glucose	100.0	+0.3
Sorbitol	100.0	+0.8

All commercial pharmaceutical formulations containing tyrosine, available in the Spanish market, contain also cysteine and/or hystidine and/or tryptophan. So, the proposed method was evaluated by analyzing two samples prepared by spiking tyrosine (10.0 mg l^{-1}). A synthetic formulation of tyrosine was prepared by solving of the analyte in physiological serum (0.9% NaCl). The obtained result was compared versus the signal obtained by injecting pure tyrosine standard solutions of 10 mg l^{-1} and a relative error of + 0.5% was found. The analyte was also determined in a more complex matrix, prepared by mixing the noninterfering 16 primary protein-forming amino acids (100 mg l^{-1}) and other 11 common excipients; namely, sodium acetate, zinc acetate, malic acid, calcium chloride, magnesium chloride, potassium chloride, sodium chloride, Na_2EDTA, sodium phosphate, glucose and sorbitol (all 100 mg l^{-1}). The obtained result was compared versus the signal obtained by injecting tyrosine standard solution of 10.0 mg l^{-1} and a relative error of + 1.8% was found.

5. Conclusions

An FIA-direct chemiluminescence method is developed for tyrosine by reaction with potassium ferricyanide, which has been employed only in four direct chemiluminescence determinations.

As a result, a new procedure showing good sensitivity, low LOD (50 μg l^{-1}), very quick (98 h^{-1}), dynamic linear range between 1.0 and 10.0 mg l^{-1} and with high reproducibility between samples and between days and solutions is proposed for determination of the amino acid. A large study on the influence of foreign compounds revealed selectivity higher than any other procedure non-based on chromatographic methods.

The analytical figures of LOD and dynamic linear range make the procedure competitive with the chemiluminescence batch procedure with potassium permanganate in sulfuric acid medium [21] and with other spectrophotometric [4,5] and fluorimetric [7,10] procedures.

In front of the existent analytical methods [1–20] (see Section 1), the main advantages of the chemiluminescent method are the simplicity, robustness and low cost of the detector, combined with the low LOD of 50 ppb. Moreover, to the former advantages must be added all those derived from the FI methodology [21].

References

[1] Y. Azuma, M. Maekawa, Y. Kuwabara, T. Nakajima, K. Taniguchi, T. Kanno, Clin. Chem. 35 (1989) 1399.
[2] I. Cudina, L.R. Joksimovic, J. Serb. Chem. Soc. 50 (1985) 457.
[3] C.S.P. Sastry, P. Satyanarayana, M.K. Tummuru, Food Chem. 17 (1985) 227.
[4] M.Z. Zou, Q.H. Ru, Z.S. Cao, G.L. Huang, Fenxi Shiyanshi 17 (1998) 61.
[5] M. Surmenian, Drug De. Ind. Pharm. 24 (1998) 691.
[6] B. Samotus, M. Leja, A. Scigalski, J. Dulinski, R. Siwanowicz, J. Sci. Food Agric. 33 (1982) 617.
[7] H. Wang, K. Du, Fenxi Huaxue 15 (1987) 864.
[8] F. Wang, Y.Z. Wu, Y. Qing, Y.X. Ci, Anal. Lett. 25 (1992) 1469.
[9] V.B. Gavrilov, A.V. Lychkovskii, E.P. Shostak, S.V. Konev, Zh. Prikl. Spektrosk. 65 (1998) 366.
[10] N.Q. Jie, J.H. Yang, X.F. Liu, R.X. Zhang, Lihua Jianyan Huaxue Fence 30 (1994) 364.
[11] G.A. Rivas, V.M. Solis, Anal. Chem. 63 (1991) 2762.
[12] F. Schubert, U. Wollenberger, F. Scheller, Biotechnol. Lett. 5 (1983) 239.
[13] G.S. Wang, T.Z. Peng, B.E. Shen, P.L. Zhu, L.M. Qu, Fenxi Huaxue 22 (1994) 590.
[14] J.C. Moreira, A.G. Fogg, Analyst 116 (1991) 249.
[15] L.Z. Wang, C.S. Ma, M.L. Zhang, Y.B. Ren, Y. Yu, Fresenius J. Anal. Chem. 351 (1995) 689.
[16] G. Ramis Ramos, A.R. Mauri Aucejo, M.C. García Álvarez-Coque, C. Mongay Fernández, Microchem. J. 36 (1987) 113.
[17] A. J. Pesce, L. A. Kaplan, (ed.) Luimica Clinica Métodos Médica Panamericana, Buenos Aires, Argentina, 1991.
[18] T.E.G. Candy, D. Mantle, J. Jones, J. Biolumin. Chemilumin. 6 (1991) 245.
[19] Y.X. Ci, J.K. Tie, Q.W. Wang, W.B. Chang, Anal. Chim. Acta 269 (1992) 109.
[20] M.E. Bolden, N.D. Danielson, J. Chromatogr. A 828 (1998) 421.
[21] J. Martínez Calatayud, Flow Injection Analysis of Pharmaceuticals. Automation in the Laboratory, Taylor and Francis, Cambridge, UK, 1996.
[22] P.J. Worsfold, A.R. Bowie, M.G. Sanders, J. Biolumin. Chemilumin. 11 (1996) 61.
[23] G.P. Chernysh, R.P. Poponina, V.I. Buntushkin, J. Anal. Chem. USSR 31 (1976) 1475.

[24] N. Grekas, A.C. Calokerinos, Talanta 37 (1990) 1043.
[25] A.B. Syropoulos, A.C. Calokerinos, Anal. Chim. Acta 255 (1991) 403.
[26] Y. Fuster Mestre, B. Fernández Band, L. Lahuerta Zamora, J. Martínez Calatayud, Analyst 124 (1999) 413.
[27] W.P. Yang, B.L. Li, Z.J. Zhang, G.H. Tian, Fenxi Huaxue 24 (1996) 579.
[28] L. Lahuerta Zamora, E. Aureccia Giménez, A. Gregorio Alapont, J. Martínez Calatayud, J. Biolumin. Chemilumin. 13 (1998) 131.
[29] A. Gregorio Alapont, L. Lahuerta Zamora, J. Martínez Calatayud, J. Pharm. Biomed. Anal. 21 (1999) 311.
[30] M.C. Sanfeliu Alonso, L. Lahuerta Zamora, J. Martínez Calatayud, Analytica Chimica Acta 437 (2001) 225.
[31] L.Q. Li, M.L. Yang, M.L. Feng, J.R. Lu, Fenxi Shiyanshi 17 (1998) 5.
[32] A.A. Alwarthan, H.A. Lohedan, Z.A. Issa, Anal. Lett. 29 (1996) 1589.
[33] J. Ouyang, W.R.G. Baeyens, J. Delanghe, G. Van der Weken, A.C. Calokerinos, Talanta 46 (1998) 961.
[34] S.L Morgan, S.N. Deming, Anal. Chim. Acta 46 (1974) 1170.
[35] H.D. Belitz, W. Grosch, Food Chemistry, second ed., Springer, Berlin, Germany, 1999, p. 69.