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Notes & Tips

Multicommuted optosensor for the determination of pipemidic acid in biological fluids

E.J. Llorent-Martínez, P. Ortega-Barrales, A. Molina-Díaz *

Department of Physical and Analytical Chemistry, Faculty of Experimental Sciences, University of Jaén, Paraje Las Lagunillas, E-23071 Jaén, Spain

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Pipemidic acid (PA),[1] 8-ethyl-5,8-dihydro-5-oxo-2-(1-piperazinyl)pyrido[2,3-*d*]pyrimidine-6-carboxylic acid, is a synthetic quinolone antibacterial agent. The antibacterial property of quinolones is associated with their potential to inhibit topoisomerase II (DNA gyrase) of bacteria. Their effectiveness depends on the quinolone structure [1]. PA is widely used for the treatment of urinary tract infections, showing high activity against both gram-positive and gram-negative bacteria.

Several methods for the determination of PA in biological fluids have been developed. Luminescence techniques are the ones used most frequently [2–6], including lanthanide-sensitized luminescence [7,8].

In terbium-sensitized luminescence, the energy absorbed by the organic chromophore (PA in this case) at its characteristic excitation wavelength is transferred to a triplet state of the molecule and then transferred intramolecularly to a resonance level of the lanthanide ion (terbium in this case), which finally emits luminescence at its particular emission wavelength. This allows analytical measurements in the phosphorescence mode by eliminating scatter, Raman, and any fluorescence background signal [9] with subsequent selectivity enhancement.

The implementation of multicommutation to flow-through optosensing combines the advantages of both methodologies: high selectivity and sensitivity, simplicity and speed, easy handling of the system, and very low consumption of sample and reagent solutions [10]. In addition, these advantages can be exploited in combination with an appropriate detection technique, providing a very selective method depending on the chosen technique.

In this work, a multicommuted flow-through optosensor for the determination of the drug in human biological fluids is developed by using terbium-sensitized luminescence as the detection technique. A luminescent terbium chelate was formed online and carried toward the sensing zone, where it developed its transitory analytical signal, as a function of the analyte concentration. This sensor could also be used for other pharmaceuticals. The same system has been tested on other quinolones as analytes with satisfactory results.

Luminescence measurements were performed with a Cary Eclipse luminescence spectrometer (Varian, Mulgrave, Australia) connected to a computer with a Cary Eclipse (Varian) software package for data collection and treatment. The phosphorescence mode was used for all of the analytical measurements.

The manifold, depicted in Fig. 1, was built using a four-channel Gilson Minipuls 3 peristaltic pump (Villiers le Bel, France) fitted with a rate selector and pump tubing type Solvflex (Elkay Products, Shrewsbury, MA, USA). An electronic interface based on ULN 2803 integrated circuits was employed to generate the electric potential (12 V) and current (100 mA) required to control the four 161T031 NResearch three-way solenoid valves (Neptune Research, West Caldwell, NJ, USA). The software for controlling the system was developed in Java. Polytetrafluoroethylene (PTFE) tubing of 0.8 mm i.d. and methacrylate connections also were used.

A Hellma flow cell 176.125 QS (25 µl inner volume and 1.5 mm light path length) was used. The cell was filled with Sephadex-SP C25 resin microbeads and was blocked at the outlet with glass wool to prevent displacement of the resin particles.

Initially, all valves are switched off and the carrier, acetic acid/sodium acetate buffer (0.15 M, pH 5.6), flows through

* Corresponding author. Fax: +34 953 212949.
E-mail address: amolina@ujaen.es (A. Molina-Díaz).

[1] *Abbreviations used:* PA, pipemidic acid; PTFE, polytetrafluoroethylene; EDTA, ethylenediamine tetraacetic acid sodium salt 2-hydrate; RSD, relative standard deviation.

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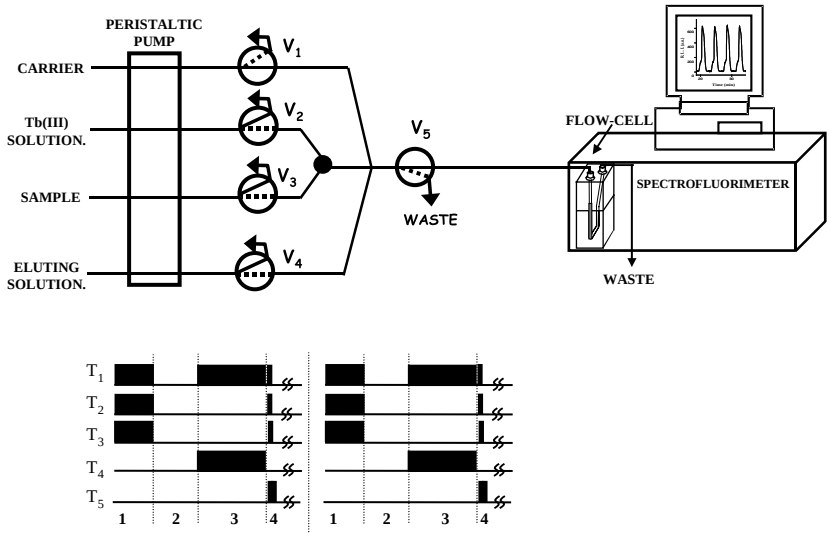

Fig. 1. Multicommuted flow configuration and valves scheme. $T_{1,2...}$ refer to the time course during which solenoid valves $V_{1,2...}$ were switched on and off. The filled rectangles above the valves' time lines indicate the times at which the corresponding valves were switched on. The steps were as follows: 1, sample introduction; 2, PA signal development; 3, PA elution; 4, cleaning step.

the flow cell while all other solutions are recycling to their respective vessels. The sample and terbium solutions are introduced and mixed by simultaneously switching on the valves V_1, V_2, and V_3 for 50 s at a flow rate of 1 ml min^{-1}.

The chelate formed online is carried toward the sensing microbeads, where it develops its transitory luminescent signal. After the maximum is reached and the elution starts, valves V_1 and V_4 are switched on and the eluting solution of 0.08 M ethylenediamine tetraacetic acid sodium salt 2-hydrate (EDTA) is introduced into the flowing system for 85 s so that the sensing support is completely regenerated. Finally, the portion of tubing still filled with sample solution is cleaned with the next sample solution to avoid any possible contamination between samples. Before the next sample is analyzed, the carrier solution flows through the sensing zone for 2 min. A typical flow profile is shown in Fig. 2.

Urine samples, collected from healthy volunteers, were spiked with a suitable PA concentration and diluted (~1:2000) with 10^{-2} M HCl before measuring.

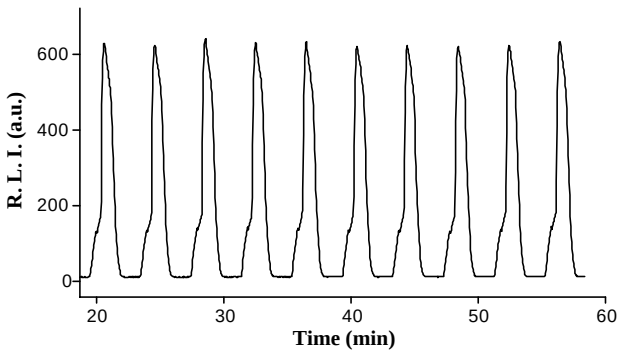

Fig. 2. Profile of the R.L.I. transient signal in the study of the repeatability of the system ($n = 10$) for a spiked urine sample containing 330 pmol ml^{-1} of PA. a.u., arbitrary units.

The Ciudad de Jaén Hospital (Jaén, Spain) kindly supplied the serum samples (obtained after coagulation and further centrifugation at 8000*g* for 5 min). The serum was spiked with an appropriate PA concentration and diluted to 10 ml with 10^{-2} M HCl. It was then filtered through a 2.7-μm pore size filter with the aid of a syringe. Finally, a suitable volume of the filtrate was diluted (~1:40) with 10^{-2} M HCl and inserted into the flowing system.

The instrumental variables were optimized for the highest net signal (complex minus terbium signals). The delay and gate time were 0.1 and 3 ms, respectively. Instrument excitation and emission slits both were set at 20 nm. The detector voltage was 600 V. The excitation and emission wavelengths were selected at 320 and 545 nm ($^5D_4 \rightarrow {}^7F_5$ transition), respectively.

A study of the response to pH and the nature of carrier and sample solutions was carried out. The signal was constant over a pH range from 2 to 7 for the carrier, but optimal repeatability was obtained over the range from 5 to 6. An acetate buffer at pH 5.6 was selected as suitable, and the optimal concentration of the buffer was chosen as 0.15 M. Maximum signal was obtained for sample at pH 2, so all sample solutions were prepared in 10^{-2} M HCl.

A study of the optimal terbium concentration (prepared in deionized water) showed that the net signal increased up to 2×10^{-3} M and then remained constant, and so 4×10^{-3} M was selected as suitable.

EDTA (0.08 M) was used to regenerate the sensing solid phase. An insertion time of 85 s of eluting solution was observed to be enough for a complete regeneration of the sensor. When EDTA is introduced into the flow system, a ternary complex of terbium, EDTA, and PA is formed. This complex, probably of an anionic nature, is not retained in the resin, thereby allowing the regeneration of the sensing support.

Under the conditions selected, the system responds linearly to PA concentrations between 19.8 and 660 pmol ml^{-1},

showing a detection limit of 5.9 pmol ml^{-1} and a relative standard deviation (RSD) of 2.1% ($n = 10$, 330 pmol ml^{-1} of PA).

The possible interference of compounds present in biological fluids was studied at a PA concentration of 250 pmol ml^{-1}. The proposed method shows a good tolerance level to species such as lactose, saccharose, urea, uric acid, potassium, calcium, sodium chlorides, and magnesium sulfate.

Determination of PA was carried out with spiked human urine and serum. The amount spiked, both in urine and in serum, was that usually found after the administration of a twice-daily dose of 400 mg [11,12] or even less. For spiked PA concentrations in serum (3.3–13.2 nmol ml^{-1}) and urine (3.3–330 nmol ml^{-1}), the mean recoveries were similar (95–105% in all cases). The average recovery significant test was used to determine the accuracy of the method.

In conclusion, for the first time, lanthanide-sensitized luminescence has been implemented as a detection technique in a multicommuted flow network. The advantages of the current system are complete automation, speed, selectivity, sensitivity, and easy handling. The determination of a widely used quinolone in complex matrices such as biological fluids is easy to carry out, showing the great potential of this detection technique. It is worth noting that the only required pretreatment is a filtration step for the serum and that no pretreatment is required in the urine samples. The sensor was also tested for other quinolone antibacterial agents, such as norfloxacin, ciprofloxacin, enoxacin, and trovafloxacin, with satisfactory results.

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